BIOLOGICAL SCIENCES CURRICULUM STUDY

Yellow Version

BIOLOGICAL SCIENCE: AN INQUIRY INTO LIFE, Harcourt, Brace & World, Inc.

Green Version

HIGH SCHOOL BIOLOGY: BSCS GREEN VERSION, Rand McNally & Co.

Blue Version

HIGH SCHOOL BIOLOGY: MOLECULES TO MAN, Houghton Mifflin Company

LABORATORY BLOCKS

The Complementarity of Structure and Function
Animal Growth and Development
Plant Growth and Development
Microbes: Their Growth, Nutrition, and Interaction
(Other titles in preparation) D. C. Heath & Company

Biological Investigations for Secondary School Students: Research Problems in Biology for the Schools, Doubleday & Company, Inc.

MATERIALS FOR THE TEACHER

Biology Teacher's Handbook, John Wiley & Sons, Inc.
Innovations in Equipment and Techniques for The Biology Teaching Laboratory, D. C. Heath & Company

BSCS Pamphlet Series, D. C. Heath & Company

CURRICULUM STUDY BULLETINS

Hurd, P. DeH. 1961. *Biological Education in American Secondary Schools 1890-1960*. BSCS Curriculum Study Bulletin No. 1, American Institute of Biological Sciences, Washington, D. C.

Brandwein, P. F., J. Metzner, E. Morholt, A. Roe, W. Rosen, 1962. *Teaching High School Biology: A Guide to Working with Potential Biologists*. BSCS Curriculum Study Bulletin No. 2, American Institute of Biological Sciences, Washington, D. C.

There are times when you have to see for yourself; it is as simple as that. Then you go to the laboratory, or into the field, or wherever you can deal with living things at first hand.

Many times, too many times, words hide mysteries—words like *Amoeba, chloroplast, chromosome.* Then the deed clarifies; the deed in the laboratory lights up the word and it becomes real because it becomes an experience.

WILLIAM V. MAYER

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BIOLOGICAL SCIENCES CURRICULUM STUDY

Initiated by the AMERICAN INSTITUTE OF BIOLOGICAL SCIENCES

STUDENT LABORATORY GUIDE

BIOLOGICAL SCIENCE:

AN INQUIRY INTO LIFE

A REVISION OF BSCS *High School Biology: Yellow Version* PREPARED BY:

JOHN A. MOORE, SUPERVISOR
Department of Zoology, Columbia University

BENTLEY GLASS
Department of Biology, The Johns Hopkins University

J. MAXWELL DAVIS
Biology Department, Bosse High School, Evansville, Indiana

WILLIAM V. MAYER
*Department of Biology, and Associate Dean,
College of Liberal Arts, Wayne State University*

DONALD H. BUCKLIN
Department of Zoology, University of Wisconsin

WILSON N. STEWART
Department of Botany, University of Illinois

GEORGE SCHWARTZ
Biology Department, Forest Hills High School, New York City

Edited by DON E. MEYER
and VIRGINIA M. DRYDEN
of the Harcourt, Brace & World *Staff*

INDIAN EDITION
NATIONAL COUNCIL OF EDUCATIONAL
RESEARCH AND TRAINING

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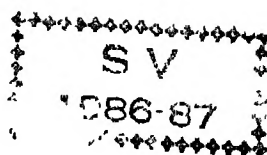
The many writers, artists, consultants, and reviewers who have contributed to experimental editions of BSCS *High School Biology: Yellow Version* are acknowledged on pages xiii-xv.

Artwork by CARU Studios

COVER PHOTOGRAPH: Courtesy of J. H. Tjio and T. T. Puck, from *Proc. Nat. Acad. Sci.*, 44, 1229 (1958). The chromosomes are shown in a stage of mitosis, arrested by colchicine. The field of the photograph has been reversed by use of a negative print.
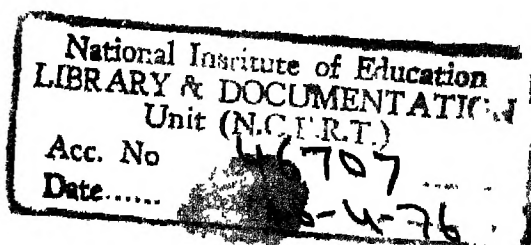
ABOUT THE INDIAN EDITION

The Biological Sciences Curriculum Study comprises a group of American biologists who are interested in improving the teaching of biology in the high schools in the United States. In order to contribute to such an improvement the BSCS has prepared, with financial support from the U. S. National Science Foundation, a variety of printed materials of which *Biological Sciences : An Inquiry Into Life* is one example.

This version (consisting of a textbook, laboratory manual and a teacher's guide) was designed for American students. The high school teachers and college professors, who were the writers, had the domestic educational pattern in mind and placed primary emphasis on the North American fauna and flora.

It follows that we do not feel that the BSCS materials are appropriate for use in the classrooms of other nations without specific modification for the local biota, customs and educational systems. We do hope that Indian colleagues will prepare such an adaptation and we would feel privileged to work with them towards such a goal.

For the interim, the National Council of Educational Research and Training have suggested to us that it would be useful to have our books reprinted in India in a limited edition so that they could be generally available to Indian scholars and teachers. Thus, interested persons could review them while weighing the advantages and disadvantages of an adaptation. We were delighted with this suggestion by the NCERT and have been pleased to do what was necessary to make this possible.

ARNOLD B. GROBMAN
Director
Biological Sciences
Curriculum Study
December 7, 1964   Boulder, Colorado, USA

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FOREWORD

With each new generation our fund of scientific knowledge increases fivefold; this remarkable growth of knowledge has led to more inadequate and more formalized science instruction. In biology, the routines of teaching have drifted ever farther from any approach that a scientist could recognize as an introduction to a science. As a consequence, there has been widespread dissatisfaction with the content and methods of our science courses in secondary schools. In January 1959, the AIBS, a professional society representing 85,000 biologists, established the Biological Sciences Curriculum Study as a means of contributing to the improvement of secondary school biological education. For the purposes of formulating basic policy, there was organized at an early date a BSCS Steering Committee (composed of college biologists, high school biology teachers, and other educators—all interested in improving the quality of the teaching of biology). Headquarters for the Study were established on the campus of the University of Colorado. Primary financial support for the Biological Sciences Curriculum Study has been provided by the National Science Foundation.

Two tasks in improving and modernizing instructional materials in biology stood out as imperative. First was the fullest possible consideration of the new perspectives in biology growing from the astonishing increase in scientific knowledge. At the current rate of scientific advance, there is about four times as much significant biological knowledge today as in 1930, and about sixteen times as much as in 1900. By the year 2000, at this rate of increase, there will be a hundred times as much biology to "cover" in the introductory course as at the beginning of the century. Obviously, then, we must be highly selective in our choice of what scientific facts, concepts, and principles to present, so that the most important developments of the new biology, together with the most profound insights of the older biology, may be included. This highly selective approach means, however, that many feasible viewpoints and approaches may claim equal validity and equally high merit. We have conse-

quently refrained from any attempt to develop a single authoritative program for the study of biology in the secondary school. Instead, we have prepared and now present a varied, balanced, and enriched program that may utilize either Yellow, Green, or Blue Versions, with the possible inclusion of any of a number of Laboratory Blocks, Invitations to Enquiry, and Special Investigations.

Secondly, we are profoundly convinced that the major fault in the teaching of biology and other sciences in the secondary schools is that emphasis has been placed on authoritative content—facts, concepts, principles—instead of being placed on the investigative processes of science and the history of scientific ideas. In order to live in a scientifically based civilization with some appreciation of the forces that are shaping the lives of modern citizens, what is especially needful is an understanding of what science really is —not a modern magic but a variety of ways of finding out verifiable information and building up concepts and principles that adequately explain what we know of nature's ways. Our primary emphasis has thus been laid upon science as investigation and inquiry. Observation, experiment, hypothesis, and verification are the four corners of this structure. The student, by personal participation in scientific processes, must come to know them and respect them. For these reasons we have eschewed the possibility of providing textbooks that could be used without the complementary laboratory programs. We have done our best to prepare, instead, a laboratory- (and field-) centered program of instruction that makes use of the textbook only as a supplement to learning. For these reasons, too, we have placed special stress on the value of the Laboratory Blocks and the Invitations to Enquiry, as well as the Special Investigations that individual students may perform. Taken together, the BSCS materials comprise a series of integrated, balanced, and enriched programs—not simply a set of independent books, films, and laboratory exercises.

Particularly important in the integration of the materials was a sort of three-dimensional frame-

work of ideas. In one dimension we determined to include a balanced consideration of microorganisms, plants, and animals, and not to ignore or slight any one of these three great subdivisions of living organisms. In another dimension we have been concerned with a balanced consideration of all levels of living organization, from the molecule through the cell, the tissue and organ, the individual, the population and species, and the community, to the entire world biosphere. In the third conceptual dimension we conceived of nine great themes that would course through all treatments of subjects and topics, extend through all levels of organization, and relate to microorganisms, plants, and animals alike. These major themes were as follows: science as investigation and inquiry; the history of biological concepts; the complementarity of structure and function; diversity of type and unity of pattern; change of organisms through time: evolution; genetic continuity; the complementarity of organism and environment; regulation and homeostasis; and the biological roots of behavior.

We were also firmly convinced that in our new courses nothing should prevent a thorough, unbiased, and scientifically objective presentation of such supposedly controversial biological subjects as organic evolution, the nature of individual and racial differences, sex and reproduction in the human species, or the problems of population growth and control. A sound biological understanding of matters such as these is the inalienable birthright of every future parent and citizen. To establish a basis for a better public understanding of the wise management of natural resources, of the biological hazards of nuclear agents in peace and in war, and of the methods by which scientific information is achieved, as primary sources of national strength and well-being in this new era of history, was clearly also an inescapable obligation of the new biology curriculum.

Perhaps the most significant feature in the development of the BSCS materials has been the fruitful cooperation between research biologists on the frontiers of science and high school teachers on the frontiers of teaching. The design and production of these teaching materials has involved a major intensive effort extending over several years.

During the summer of 1960, the BSCS assembled a group of 70 high school biology teachers and university research biologists to prepare preliminary trial materials. The books produced were tested in approximately 100 schools throughout the country, and based on that classroom experience the materials were thoroughly revised during the summer of 1961. The revised trial materials were then used in 500 schools during the 1961-62 academic year and in 950 schools the following year. The books now available reflect this very extensive experience with trial editions. The BSCS appreciates the singularly important contributions to the improvement of biological education made by over 1,000 teachers and 150,-000 students through their use of the trial instructional materials.

Each one of the three versions, as we have said, has its own flavor and thematic approach. One of these versions, known during the three-year trial period as the Yellow Version, has evolved into the present materials. The rationale underlying the development of the Yellow Version is given in the textbook Preface by Dr. John A. Moore, who has served as supervisor of the writing team that developed the Yellow Version.

Hundreds of the nation's biological scientists and teachers have worked diligently on the BSCS materials. We hope that high school students and their teachers will continue to find that these efforts have been of value.

BENTLEY GLASS, CHAIRMAN, BSCS
The Johns Hopkins University
Baltimore, Maryland

ARNOLD B. GROBMAN, DIRECTOR, BSCS
University of Colorado
Boulder, Colorado

February 1, 1963

PREFACE

While this *Student Laboratory Guide* is a product of the combined efforts of many individuals over a period of years, this final revision has been greatly aided by a selected few. On the basis of trials throughout the United States since 1960, and the combined experience of hundreds of teachers and thousands of students using these materials in an experimental version, the final writing conference was able to draw upon the combined experience of teachers and students alike to produce this laboratory manual.

Mr. J. Maxwell Davis of Bosse High School, Evansville, Indiana, Mr. George Schwartz, Forest Hills High School, New York, and Mr. Russell C. Oakes, Huntington High School, New York, have in this final revision, offered critical suggestions of great value.

The exercises on botany have been completely revised by Dr. Wilson Stewart of the Department of Botany of the University of Illinois at Urbana, Illinois. Dr. Donald Bucklin, of the Department of Zoology of the University of Wisconsin at Madison, Wisconsin, rewrote those exercises concerned with animal development and several of those dealing with cells.

In his supervisory capacity Dr. John A. Moore was of inestimable help. To Dr. Bentley Glass of The Johns Hopkins University, Chairman of the BSCS, goes an especial acknowledgment for his contributions in the exercises on cells, and his complete reworking of all the exercises on genetics and evolution. In addition, Dr. Glass critically read and commented on all of the laboratory exercises.

While wishing to acknowledge the contributions of each of the above to this *Student Laboratory Guide*, decisions ultimately fall to one individual. Therefore, for any errors or sins of omission or commission, the undersigned is directly culpable as the preparator of the final manuscript.

WILLIAM V. MAYER
SENIOR EDITOR
Student Laboratory Guide, Yellow Version

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Henry M. Wallbrunn, University of Florida, Gainesville, Florida, 1961

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Robert L. Wistort, High Point High School, Hyattsville, Maryland, 1961

Louise A. Wolf, New York City, 1962, 1963

Delaphine G. R. Wyckoff, Wellesley College, Wellesley, Massachusetts, 1960

Scientific societies that have reviewed experimental editions—and the members of the societies who undertook these reviews—include:

AMERICAN ACADEMY OF MICROBIOLOGY:
Dr. Perry Wilson (Chairman), University of Wisconsin; Dr. Raymond Doetsch, University of Maryland; Dr. Neal Groman, University of Washington; Dr. Michael J. Pelczar, University of Maryland; and Dr. Wayne W. Umbreit, Rutgers University

AMERICAN GENETIC ASSOCIATION:
Dr. Ralph Singleton (Chairman), University of Virginia; Dr. F. B. Hutt, Cornell University; Dr. D. T. Morgan, University of Maryland; and Dr. Marcus Rhoades, Indiana University

AMERICAN PHYSIOLOGICAL SOCIETY:
Dr. J. R. Brobeck (Chairman), University of Pennsylvania; Dr. Arthur W. Martin, University of Washington; and Dr. R. R. Ronkin, University of Delaware

AMERICAN PHYTOPATHOLOGICAL SOCIETY:
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AMERICAN SOCIETY FOR HORTICULTURAL SCIENCES:
Dr. O. W. Davidson (Chairman), Rutgers University; Dr. Russell Eggert, State University of New Hampshire; and Professor A. F. DeWirth, Agricultural and Mechanical College of Texas

AMERICAN SOCIETY OF HUMAN GENETICS:
Dr. Arthur Steinberg (Chairman), Western Reserve University

AMERICAN SOCIETY FOR MICROBIOLOGY:
Dr. H. J. Blumenthal (Chairman), University of

Michigan Medical School; Mr. Byron Bernard, La Porte High School, La Porte, Indiana; Dr. Arthur R. Colmer, Louisiana State University; Dr. Arnold L. Demain, Merck, Sharp and Dohme; Dr. S. G. Knight, University of Wisconsin; Dr. L. S. McClung, Indiana University; Dr. W. J. Walter, Montana State College; and Dr. E. D. Weinberg, Indiana University

AMERICAN SOCIETY OF PARASITOLOGISTS:
Dr. Norman Levine (Chairman), University of Illinois; Dr. David R. Lincicome, Howard University; Dr. O. Wilford Olsen, Colorado State University; and Dr. Marietta Voge, University of California

AMERICAN SOCIETY OF PLANT TAXONOMISTS:
Dr. C. Ritchie Bell (Chairman), University of North Carolina; Dr. L. Constance, University of California at Berkeley; Dr. Mildred Mathias, University of California at Los Angeles; Dr. J. Proskauer, University of California at Los Angeles; Dr. A. E. Radford, University of North Carolina; and Dr. Peter Raven, Rancho Santa Ana Botanic Garden

AMERICAN SOCIETY OF ZOOLOGISTS:
Dr. Carl Gans, University of Buffalo; Dr. Clarence Goodnight, Purdue University; Dr. Ronald R. Novales, Northwestern University; and Dr. J. Paul Scott, Jackson Memorial Laboratory

BIOPHYSICAL SOCIETY:
Dr. Richard S. Bear (Chairman), Boston University; and Dr. N. A. Coulter, Jr., Ohio State University

BOTANICAL SOCIETY OF AMERICA:
Dr. Adolph Hecht, Washington State University; Dr. S. N. Postlethwait, Purdue University; and Dr. John Thomson, University of Wisconsin

COUNCIL FOR EXCEPTIONAL CHILDREN:
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DEPARTMENT OF RURAL EDUCATION:
Mr. Fred Guffin, Fulton County Board of Education, Hapeville, Georgia

ECOLOGICAL SOCIETY OF AMERICA:
Dr. Frank Golley (Chairman), University of Georgia; and Dr. Paul Johnsgard, University of Nebraska

ENTOMOLOGICAL SOCIETY OF AMERICA:
Dr. Robert V. Travis (Chairman), Garden Pest Control; and Mr. George Senechal, Valentine City Schools, Valentine, Nebraska

GENETICS SOCIETY OF AMERICA:
Dr. David Nanney (Chairman), University of Illinois; Dr. Robert Edgar, California Institute of Technology; and Dr. E. S. Russell, Roscoe B. Jackson Memorial Laboratory

NATIONAL ASSOCIATION OF BIOLOGY TEACHERS:
Dr. Edward Frankel, Bureau of Educational Research, New York City Board of Education; Mr. William M. Smith, Thomas Carr Howe High School, Indianapolis; and Mr. Donald Winslow, Indiana University

NATIONAL ASSOCIATION FOR RESEARCH IN SCIENCE TEACHING:
Dr. W. C. VanDeventer (Chairman), Western Michigan University; Dr. Ralph P. Frazier, Em-

poria State Teachers College; and Dr. Joseph Novak, Purdue University

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Rev. Mark H. Bauer, S.J. (Chairman), Georgetown University; Brother Bernardine, C.F.X., Archbishop Stephinac High School, White Plains, New York; Rev. Donald M. Chigar, Mount Carmel High School, Houston; Sister Mary Ivo, B.V.M., The Immaculata, Chicago; Sister Julia Marie, O.S.F., Holy Family College, Manitowoc, Wisconsin; and Rev. George A. Walsh, O.S.F.S., Cathedral Prep, Erie, Pennsylvania

NATIONAL SCIENCE TEACHERS ASSOCIATION:

Dr. Darwin Levine (Chairman), New York City Board of Education; Mrs. Leona Adler, New York University; Mr. Thomas Lawrence, Erasmus Hall High School, Brooklyn; Mr. Kenneth Meyer, Harrison, New York; Mr. Jesse Miller, Manhasset Public Schools, Long Island; and Mr. Robert Weinberger, Flushing High School, New York

POULTRY SCIENCE ASSOCIATION:

Dr. Walter C. Morgan (Chairman), South Dakota State College; Dr. D. W. MacLaury, University of Kentucky; Dr. George D. Quigley, University of Maryland; Dr. E. A. Shano, Cornell University; and Dr. D. W. Talmage, University of Connecticut

SOCIETY OF GENERAL PHYSIOLOGY:

Dr. R. R. Ronkin (Chairman), University of Delaware

SOCIETY FOR INDUSTRIAL MICROBIOLOGY:

Dr. John N. Porter (Chairman), Lederle Laboratories; Dr. Marlin A. Espenshade, Wyeth Laboratories, Inc.; and Dr. B. Malin, Eli Lilly and Company

SOCIETY FOR THE STUDY OF EVOLUTION:

Dr. John S. Mecham (Chairman), Auburn University; Dr. David J. Merrell, University of Minnesota; Dr. Robert Sokal, University of Kansas; and Dr. Robert K. Vickery, University of Utah

SOCIETY FOR THE STUDY OF GROWTH AND DEVELOPMENT:

Dr. Arthur Galston (Chairman), Yale University; and Dr. Robert L. DeHaan, Carnegie Institute of Washington

Individuals who reviewed experimental editions include:

Mr. Wilbur Pieter Bijhouwer, Chicago, Illinois

Dr. W. D. Billings, Duke University

Dr. Robert E. Bills, University of Alabama

Dr. H. J. Blumenthal, University of Michigan Medical School

Dr. Harold C. Bold, University of Texas

Mr. Ernest C. Borden, Phillips Exeter Academy

Dr. J. K. Brierley, British Ministry of Education

Dr. J. A. Campbell, Chemical Education Material Study

Mr. L. C. Comber, British Embassy

Dr. Henry S. Conard, Grinnell College

Dr. Harriet Creighton, Wellesley College

Dr. R. F. Dawson, Columbia University

Dr. Fred D. Emerson, Elon College

Miss Janice B. Footlik, Peoria, Illinois

Mr. S. E. Frederick, Denver, Colorado

Dr. Shelby Gerking, Indiana University

Dr. Ira Gordon, University of Florida

Dr. Rollin D. Hotchkiss, The Rockefeller Institute

Dr. J. Gordon Kaplan, Dalhousie University, Halifax, Nova Scotia, Canada

Dr. Harlan Lewis, University of California

Professor James W. McFarland, Air Force Academy

Dr. Lindsay Olive, Columbia University

Dr. Leonard Ornstein, The Mount Sinai Hospital

Dr. Dominick J. Paolillo, University of Illinois

Dr. Arnold Ravin, Rochester University

Dr. Isaac Schlectmeister, Southern Illinois University

Dr. Ray F. Smith, University of California

Dr. G. Ledyard Stebbins, University of California

Dr. Carl P. Swanson, The Johns Hopkins University

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Dr. Jacob Uhrich, Trinity University

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Cells

The following exercises may serve to introduce many of you to laboratory work. Not only will you be learning how to work in the laboratory, but you also will be learning how a scientist works.

The basic tools of biology will be introduced and you will learn to use them as you progress through the laboratory course.

You will be concerned not so much with whether an organism is a plant or an animal or a microorganism, but rather with the basic structures and functions common to all living things, many of which will be studied later. You will come to understand that the cell is the structural and functional unit of all living organisms and that it is a highly complex system performing functions far beyond what you might expect anything of its size to accomplish. We cannot help but wonder at the very fine organization of the living substance that allows such a wide variety of activities to be carried out in such a compact form.

A BIOLOGICAL PROBLEM—

MALARIA

Chapter 1 of the textbook deals with malaria as a problem in biology, and scientific methods are emphasized. Because the chapter has undoubtedly raised a number of questions in your mind concerning malaria, you probably would like more information about it. This demonstration is designed to provide you with a general but not highly detailed familiarity with the life history of *Plasmodium vivax*, the one-celled organism which is the cause of human malaria.

■ The purpose of this exercise is to give you a general outline of the life cycle of *Plasmodium* as a background for the textbook discussion of malaria as a problem in biological research.

MATERIALS

Demonstrations of malarial parasites
Slides, charts, pictures, and other demonstration materials

PROCEDURE

Observe the demonstrations as directed by your teacher. On the basis of your observations of the malarial parasite, why was the development of the microscope to a high degree essential before the life cycle could be understood? (1)

The life cycle of the malarial parasite, *Plasmodium vivax*, is shown in Figure 1-1-1. Study this life cycle carefully and answer the following questions.

Suppose you made the observation that a mosquito bit one human being and then another but the second individual did not get malaria: How would you explain this? (2)

In which of the following situations would malaria be transmitted from an infected person to a noninfected person? (3)

a. Using a towel which has been used by an individual with malaria?

b. Eating off the same dishes as a person who has malaria?

c. Wearing the same clothes as a person with malaria?

d. Being kept in the same room and breathing the same air as a person with malaria?

What would happen if the parasites entered every red blood cell of the human body and broke out simultaneously? (4)

In a mosquito which has bitten an infected person, parasites move from the intestine to the salivary glands. What effect would there be on the life cycle if the parasites did not move to the salivary glands? (5)

At what step in the diagramed life cycle of the malarial parasite could the life cycle be most easily broken? (6)

On November 24, 1961, intramuscular injections of a new drug (C1501) were given to five volunteers at the United States penitentiary in Atlanta. About a week later the volunteers were allowed to be bitten by mosquitoes heavily infected with malaria. If this drug were effective against malaria, what would you expect the results of this experiment to be? (7)

Would the evidence from the five volunteers be conclusive regarding action of the drug C1501? (8)

As described above, what does this experiment lack to provide an adequate test? (9)

Properly performed experiments reported on November 1, 1962, seemed not only to show that the drug prevented malaria but also that it cured the disease. In what way would C1501 be an improvement over the cinchona bark mentioned in your textbook? (10)

It has been estimated that malaria affects 200 million persons each year, killing 2 million of those so affected. In 1961, however, only 73

Intestine

Salivary gland

G. Parasites enter stomach of mosquito and later move to salivary glands.

A. Infected mosquito bites uninfected man. Parasites injected into human blood vessel in mosquito saliva.

F. Uninfected mosquito bites infected man, taking up red blood cells containing parasites.

Skin

B. Parasites are carried in blood to liver and enter liver tissue.

Blood vessel to liver tissues

Parasites enter other red blood cells

E. Red blood cells burst, releasing parasites (bursting and fever occur every 48 hours).

C. Parasites leave liver tissue and enter red blood cells.

D. Parasites multiply in red blood cells.

Figure 1-1-1   Life cycle of malarial parasite.

cases of malaria were reported in the United States. How do you account for the few malarial cases in the United States out of 200 million throughout the world? (11)

Compare the scientific methods used to determine life cycles of the organisms causing sleeping sickness, wheat rust, or yellow fever with those described for malaria. (12)

EARLY EXPERIMENTS

IN SPONTANEOUS GENERATION

Even though Leeuwenhoek discovered microorganisms (yeasts, bacteria, and other one-celled organisms) in the latter half of the seventeenth century, it was nearly two hundred years before the study of microorganisms and their activities received much attention from the scientific world. The delay was due in part to the lack of adequate methods of observation and study. With the development and use of the optical microscope there was a growing belief that microorganisms were connected with the putrefaction (rotting) of meat and the fermentation of fruit juices. Scientists and philosophers speculated on the causes of putrefaction and fermentation. Likewise, there was speculation on the origin of the microorganisms observed in fermenting juices and putrefying infusions. Chapter 2 in the textbook discusses the question about the origin of life through spontaneous generation and should be read before doing this exercise.

Do microorganisms cause fermentation and putrefaction, or are they the result of these processes?

Do microorganisms arise from the nonliving materials; that is, do they appear spontaneously? Or do they come from pre-existing microorganisms? And if so, how? Such questions led scientists into a heated controversy about the spontaneous generation of yeasts, bacteria, and other microorganisms. This battle was raging when Louis Pasteur was doing his work on fermentation a century ago. Because microorganisms are so small that they cannot be seen individually, with the unaided eye, it is difficult to answer these questions by simple observation.

At the time of the American Revolution, an Italian, Lazzaro Spallanzani, had conducted many experiments with hay infusions in an attempt to answer these questions—specifically, to test the popular theory of spontaneous generation. But his results and his interpreta-

tion of them failed to convince those who believed in spontaneous generation. Later, Louis Pasteur, seeking the answers, carried out carefully designed experiments with yeast and sugar which he performed in connection with his public lectures. His experiments provided more decisive answers.

■ The purpose of this demonstration is to visualize the work of Spallanzani and Pasteur.

MATERIALS

500 ml nutrient broth of beef bouillon
Seven flasks (250 ml)
Straight glass tube (7-8 mm diameter) 8-10 cm long
C-shaped glass tube (7-8 mm diameter) 14-16 cm long
S-shaped glass tube (7-8 mm diameter) 18-20 cm long
Two cork stoppers for flasks, three cork stoppers with holes to receive glass tubes
Screen or gauze
Sealing wax or paraffin
Graduated cylinder (100 ml)

PROCEDURE

Put 70 milliliters (ml) of the broth into each flask.

Treat the flasks as follows:

Flask 1: Overall control
Plug with cork stopper and seal the cork to the flask with warm wax or paraffin. Do not heat.

Flask 2: Spallanzani's control
Boil gently for 15 minutes. Leave open.

Flask 3: Spallanzani's experiment
Boil gently for 15 minutes. After heating, close with a cork. Seal with wax or paraffin.

1. Unheated
___ days

2. Boiled
___ days

3. Boiled
___ days

4. Autoclaved
_____ days

5. Autoclaved
_____ days

6. Autoclaved
_____ weeks
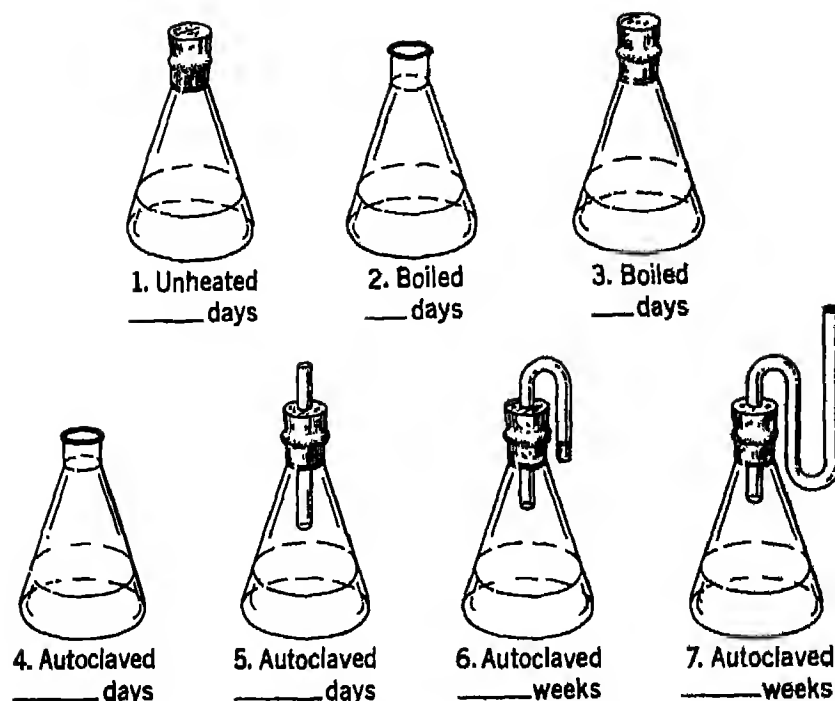
7. Autoclaved
_____ weeks

Figure 2-1-1 Spontaneous generation.

Flask 4: Pasteur's control

Heat in the pressure cooker or autoclave for 15 minutes at 15 lbs pressure. Leave unplugged.

Flask 5: Modified Pasteur's control

Plug with a cork stopper through which an open, straight glass tube has been inserted. Heat in the pressure cooker or autoclave as for Flask 4. Then seal cork with wax or paraffin.

Flask 6: Pasteur's first experiment

Plug with a cork stopper through which the C-shaped glass tube has been inserted. Heat in pressure cooker or autoclave as for Flask 4. To seal the exposed end of the C-tube, heat gently and plug with wax or paraffin. Also seal the cork to the flask.

Flask 7: Pasteur's final experiment

Plug with a cork stopper through which the S-shaped tube has been inserted. Heat in the pressure cooker or autoclave as for Flask 4. Seal the cork to the flask with warm wax or paraffin.

Put all the flasks on a laboratory table (not in direct sunlight or over a radiator).

Look for changes in the flasks, at first from day to day, later on from week to week. When any changes in the appearance of the flasks have occurred, record them and also note whether any odor is present in those which are open. Do not remove any corks, however, as that would, of course, spoil the experiment.

Record your observations either on the accompanying diagram (Figure 2-1-1) or a similar diagram. Show changes by shadowing the contents of the flask and state in the spaces under the flasks the number of days or weeks before changes appear. (1)

From the observations you have made on the contents of the flasks, what are your conclusions about the origin and sources of microorganisms in nutrient broth? (2)

Why is it important to leave Flasks 2 and 4 unplugged? (3)

What is the purpose of the S-shaped tube in Flask 7? (4)

Why was Pasteur's experiment more successful than Spallanzani's? (5)

What argument might be brought by an abiogenist against sealing Flask 6? (6)

What practical aspects of this experiment can you suggest? (7)

USING

THE COMPOUND MICROSCOPE

The microscope is an instrument especially designed for the study of objects too small to be seen and examined with the unaided eye. Your microscope will act as an extended sense of sight, reaching down into a new world—the world of the very small—to reveal what is otherwise invisible.

In our work with living organisms we will either use or observe types of microscopes with various powers of magnification—from the stereoscopic dissecting microscope magnifying from 4 to 40 times to the electron microscope which enlarges images more than 100,000 times. Usually, however, we will work with a student-type compound microscope magnifying 100 to 440 times.

The microscope is the most important tool you will use in biology, and its mastery at this time will make much of the following work easier and more meaningful to you.

■ The purpose of this exercise is to introduce you to the compound microscope and to give you practice in its use.

MATERIALS (Parts A, B, C, and E)

Compound microscope
Lens paper
Several standard glass microscope slides
Round or square cover glasses
Soft cloth free of loose fibers
Medicine dropper (pipette)
Nylon or other cloth
Dissecting needle
Fibers of cotton, wool, and human hair
Fine-print newspaper containing the letter "e"
Tumbler of water

PROCEDURE

Part A:
Setting Up the Microscope

1. When taking the microscope from its case, carry it with both hands. Hold the arm with one hand, and place the other hand under the base. Set the instrument down gently, the arm toward you, the stage away from you, and the base several inches from the edge of the table.

2. Locate on your microscope the parts shown in Figure 3-1-1.

3. Learn the names of all the parts in the drawing, as we shall refer to them often throughout the course of our work in the laboratory.

4. Rotate the nosepiece so that the low-power objective (the shorter one) is in line with the body tube. It should click or snap into position.

5. If your microscope has a mirror instead of a substage illuminator, you must look through the ocular and move the mirror around until it reflects light upward through the opening in the stage. Use the flat side of the mirror, not the concave side. Do not let direct sunlight strike the mirror; the illumination would be too bright.

6. Still looking through the ocular of the microscope, adjust the mirror, if any, and the diaphragm (by its arm or diaphragm disk) so that the round field of view is evenly illuminated, without glare.

In those microscopes which are provided with a condenser, the diaphragm is a part of the condenser. The condenser is a device whereby the light can be critically focused on the specimen on the stage.

7. If the ocular or objective is cloudy or dusty, wipe the lenses gently in one direction with a piece of lens paper. Do *not* use any other kind of paper or cloth. Use a piece of lens paper only once.

THE COMPOUND MICROSCOPE

OCULAR
Contains lenses to increase magnification; it may be replaced with another of lower or higher magnification.

BODY TUBE
Holds lenses of ocular and objectives at the proper working distance from each other.

COARSE ADJUSTMENT
Moves body tube up and down approximately to the correct distance from the specimen.

ARM
Supports body tube and coarse adjustment.

NOSEPIECE
Permits interchange of low- and high-power objectives.

OBJECTIVES
Contain lenses of different magnifications; here the shorter, low-power objective is 10X, the longer, high-power objective is 43X.

STAGE
Supports slide over hole that admits light from mirror below.

DIAPHRAGM
Regulates amount of light passing through the specimen.

STAGE CLIPS
Hold specimen slide firmly in place.

BASE
Firm support bearing weight of microscope.

MIRROR
Reflects light upward through diaphragm and hole in stage.

INCLINATION JOINT
Permits tilting to adjust to eye level.

FINE ADJUSTMENT
Permits exact focusing by moving stage or body tube up or down very slightly.

Figure 3-1-1

Part B:

How to Prepare Materials

Materials to be studied under the microscope are placed on a standard glass microscope slide. Generally the material on the slide is covered with a thin round or square piece of glass or plastic usually called a **cover glass**. Both slide and cover glass should be as clean as possible.

Slides and cover glasses should always be held by their edges (Figure 3-1-2). Clean the glass slide with water, while holding the slide by its edges with your fingers. Wipe clean and dry with a soft cloth free of loose fibers or lint. *A cover glass is fragile! Handle with care!* Clean both sides of the cover glass simultaneously, using the soft cloth and being careful not to touch the surfaces of the glass with your fingers.

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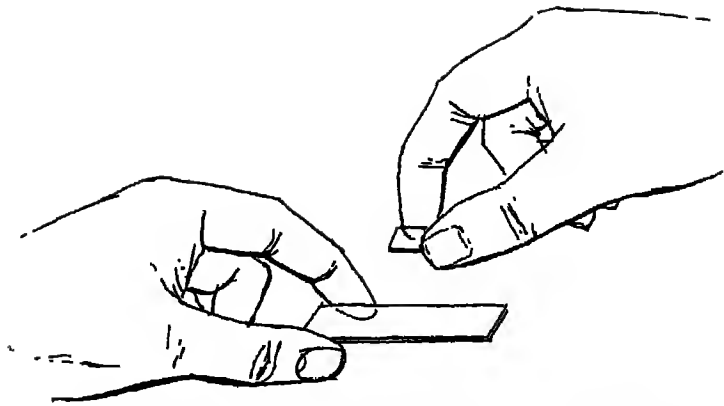
Figure 3-1-2 How to handle slides and cover glasses.

Cut or tear a piece of newspaper bearing fine print on which the small letter "e" appears, into a piece about 1 cm across.

Place this piece of newspaper on the center of the slide, with the letter "e" right side up.

With a medicine dropper or pipette put *one* drop of water on the piece of paper.

After waiting a few moments to allow the water to soak into the paper, put the cover glass over the paper. It requires some skill to place the cover glass on the slide so that no air bubbles are produced. The best method is to hold the cover glass at approximately a 45° angle to the slide and gently lower it with a dissecting needle until it covers the water, as shown in Figure 3-1-3. Bubbles may be removed by a gentle tap on the cover glass with the eraser end of a pencil. You have now prepared a **wet mount**, or water mount, of the piece of paper.

Part C:
How to Focus the Microscope

1. Place the slide you have prepared on the stage of the microscope so that the paper is centered above the opening in the stage.

2. While looking at the microscope *from one side*, use the coarse adjustment to lower the body tube until the low-power objective almost touches the cover glass or until the stop is reached that prevents the objective from being lowered any farther. Never lower the body tube while looking through the eyepiece—you may accidentally ram the objective into the slide, breaking it and damaging the very expensive lens of the objective.
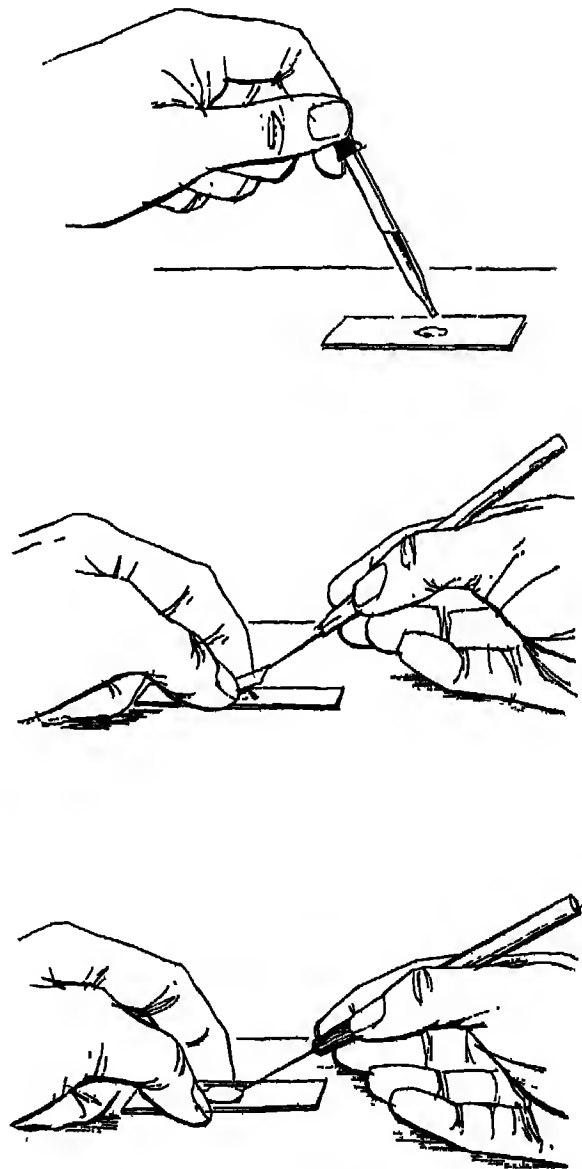
Figure 3-1-3 Preparing a wet mount.

3. While looking through the ocular, keep both eyes open. This will be difficult at first, but you can soon learn to ignore what is seen by the eye not looking through the microscope. Squinting and closing one eye may produce eyestrain. Learn *to apply the left eye to the microscope if you are right-handed, the right eye if you are left-handed. This makes it easier to draw as you look through the microscope.*

4. While looking through the ocular, use the coarse adjustment to raise the body tube until the printed letter comes into view. With the fine adjustment, make the focus as sharp as possible. What is the position of the letter "e" as seen through the microscope? (1)

Now move the slide on the stage from right to left. Which way does the image move? (2) Move the slide away from you. Which way does the image move? (3)

Prepare another wet mount as directed in Part B above, this time using a small piece of nylon (or other) cloth. Observe the cloth under low power, again following the same steps in focusing above.

Now observe the cloth under high power as follows. Move the object on the slide to the center of the field. Unless your instructor tells you that your microscope is parfocal, first raise the body tube and then change from the low-power objective to the high-power one. This must *always* be the procedure when using microscopes that are not parfocal. (With parfocal microscopes both the low- and high-power objectives are adjusted to the same focus, so you may simply switch from low power to high power without raising the tube.) Repeat the second and third steps in focusing. Be sure that the high-power objective does not touch the slide. Never turn the coarse adjustment to move the high-power objective downwards; use only the fine adjustment—and be sure to use it cautiously!

Does the change from low to high power change the position of the image in the field of view? (4) Does the field of view show a larger or smaller area of the object? (5) Is the brightness of the field greater or less than with low power? (6) Adjust the diaphragm to restore an even illumination without glare.

The cloth is thicker than the paper with the letter "e," so you must rotate the fine adjustment knob back and forth slowly to see the fibers at different depths. Thus you can get a three-dimensional picture of the cloth: length, width, and also depth. If time permits, you will also find it interesting to look at fibers of cotton, wool, and human hair.

Part D:
Care of the Microscope

The microscope, being an expensive instrument, must be given proper care. Again, it should always be carried by the arm and in an upright position with one hand under the base. When placed on the table it should be several inches from the edge in order to prevent an accidental fall to the floor. Avoid tilting the instrument at the inclination joint unless absolutely necessary because of your height. If the microscope is tilted and if you are using a temporary slide made with a drop of water, the material and water may run off the slide. However, if it is necessary to use it in a tilted position, never leave it tilted when not in use, as the instrument is not well balanced then. Before and after use, the lenses should be cleaned with lens paper. Do not use any other material for cleaning lenses, as the optical glass is soft and easily scratched.

At the end of a laboratory period turn the low-power objective into position and adjust it approximately 1 cm (about half an inch) above the stage. If the microscope has been tilted, restore it to the upright position. Be sure the stage clips do not extend beyond the stage. Return the microscope to the proper storage place. Always clean all slides and cover glasses which you have used.

Part E:
Magnification and Resolving Power

It might seem logical to you that the more we might magnify the image of a cell, the more we could see in it. But this is not true.

Let us first consider how objects are magnified. Light rays passing through the curved surfaces of transparent substances, such as glass or water, are bent. It is this bending of light rays that produces an enlarged image.

You can observe the magnifying effect of curved surfaces by a simple experiment. Hold this page in a vertical position about 3 cm from a tumbler full of water. Now look at the page through the tumbler and you will see that the words are enlarged.

The observation that pieces of curved glass will magnify is probably as old as glass itself. Lenses that could magnify objects so that they appeared about twenty times their actual size were perfected during the Middle Ages. The simplest type of magnifying instrument is a

reading glass, a doubly convex piece of glass, which will magnify objects to two or three times their normal size (Figure 3-1-4). The marvelous microscopes made by Antony van Leeuwenhoek in the seventeenth century were all simple, single lenses like a reading glass, but so cleverly ground that they revealed a new world of life (see Figure 2-6 in Chapter 2 and Figure 10-1a in Chapter 10 of the textbook).

About 1600 (shortly before the first settlers arrived in Virginia), a new principle of enlarging was discovered, that of double magnification. It was learned that the magnified image formed by one lens could be magnified again by a second lens. The first crude instrument of this type consisted of a tube with a magnifying lens at each end. This was the first compound microscope.

We have already seen how Robert Hooke constructed an improved version of the microscope and saw cells for the first time. Throughout the seventeenth and eighteenth centuries the instruments became better and better. Early in the nineteenth century it became possible to observe the nucleus with relative ease. The instruments and the methods of preparing cells for observation, however, were not yet perfected to the point where biologists could discover mitochondria, Golgi bodies, and chromosomes. The lack of proper techniques prevented any further advance at that time in the understanding of the structure of cells.

In the decades from 1870 to 1900, greatly improved methods for fixing, cutting, and staining cells were developed. In addition, the compound research microscope reached such a high degree of perfection that all later improvements in it have been relatively minor.

This perfected compound microscope gave a useful magnification of about 1500 times natural size. It revealed chromosomes, mitochondria, cell membranes, and chloroplasts. These and many other structures were the identifiable components of cells. But what was *their* internal structure? The compound microscope revealed very few internal details. Any striking progress in our understanding had to await the development of new methods and new tools. How would it be possible to improve the microscope still more?

The answer seems easy. If the best research-type compound microscope can magnify an object only 1500 or possibly 2000 diameters, then we must make a better one. Unfortunately we simply cannot. There is no way of greatly improving the compound microscope that we now use. As a matter of fact, a biologist using
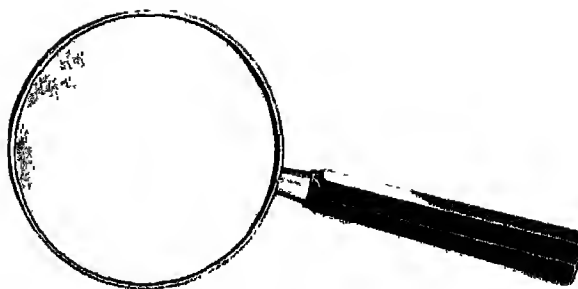
Figure 3-1-4 A hand lens, or reading glass.

the best microscopes available in 1900 could see as much in a cell as we can see with modern compound microscopes.

The limiting factor is not man's inability to build a better compound microscope, but the nature of light itself. Equally important with magnification in a microscope is its resolving power—the power to see separate images of objects that fall very close together on the eye.

The distinction between magnification and resolving power can be made clear by recalling a common experience—that of observing the headlights of an automobile approaching at night. If the automobile is a very long distance away, the two headlights appear as one (Figure 3-1-5A). If you took the picture of the headlights with a camera lens equivalent to the lens of the eye and then enlarged the picture as much as possible, there would still appear to be only one light (photograph B). Magnification of the picture has not separated the one light into two. Even a tenfold enlargement of the picture would show only one spot of light because the photographic negative would have only one spot to enlarge. A good ten-power telescope, however, would show that the distant automobile has two headlights (photograph C).

The problem for the eye, camera, and telescope is to reveal whether there is really one object or two. This ability to discriminate depends on the resolving power. In the case of the distant headlights, the eye and camera fail to detect a space between them. This is a limitation of the eye and the camera, because of the nature of light.

Poor resolving power was also a limitation of most simple lenses and of early models of the compound microscope. By the end of the nineteenth century, however, the compound microscope had about reached the theoretical limit of its resolving power. The compound microscope could not be made to show any more details even when its magnification was increased.

But what determines the limit of resolving power? It is the nature of light itself. In general, two objects will always appear as one if they are separated by a distance less than half the wavelength of the light being used, or if the distance between the images of the object on the retina of the eye or on the photographic film or plate is that small.

It may come as a surprise to you that light has length. In some respects light behaves as though it travels in waves. Physicists have measured the distance between waves, so we can say that these waves have *lengths*. White light, such as that from the sun, is a mixture of light of many wavelengths. When white light is passed through a prism, a spectrum of all colors is formed—red, orange, yellow, green, blue, and violet (see Figure 15-4 in the textbook). The shortest wavelength that can be detected by the human eye is that of violet light, a wavelength of about 400 millimicrons (mμ). The longest wavelength we can detect is that of red light, about 650 mμ. Thus the spectrum of light visible to the human eye extends from about 400 mμ to about 650 mμ. The eye is most sensitive to green light, which is about 500 mμ in wavelength. No object much smaller than one-half this distance (or 250 mμ) can be seen with a light microscope. When two objects are less than this distance apart, they cannot be resolved by the light microscope.

Since the human eye cannot ordinarily resolve objects separated by less than 0.1 millimeter (mm) (100,000 mμ), a microscope can extend the resolving power of the eye about 400 times. (Ultraviolet light, which cannot be seen by our eyes at all but which can be used to make photographs, and which has a wavelength less than that of visible light, can therefore be used to "see" what cannot be seen.) From the wavelength of *violet* light, calculate the finest resolution the eye can theoretically perceive through the microscope. (7)

In summary, a microscope does two things: first, it provides magnification; second, it permits us to see objects as separate images that are very close together and would appear as a single object to our eyes. The ability to see through the microscope objects of smaller sizes than can otherwise be seen depends both on the magnification and the resolving power, but more on the latter than the former.

A

B

C

Figure 3-1-5 **Resolution versus magnification.**

MICROSCOPIC MEASUREMENTS

The microscope can be used not only to observe very small objects, but also to measure their sizes. Indeed, the microscope is an important measuring instrument in biological science. Microscopic measurements are expressed in units of the metric system, which is a decimal system based on the meter.

■ The purpose of this exercise is to learn to use the metric system for making measurements of very small objects with the aid of the microscope.

MATERIALS (Parts A and B)

Compound microscope
Meter stick
Plastic ruler marked in millimeters
Optical micrometer (optional)
Slide and cover glass
Pipette
Filamentous algae or other cells or objects
 for estimating diameters

PROCEDURE

Part A:

Use of the Metric System

You probably have had little experience with the metric system of measurements. You are accustomed to using such units as feet, pounds, and quarts, which are a part of the English system of measurements. The English system is used only in Great Britain and the United States, and the units of length, weight, area, and volume are not related to each other in any simple manner. All other civilized countries use the metric system, which is a decimal system like the monetary system of the United States. In order, therefore, to use a simpler, more coherent, and more universal system, all measurements in scientific studies are expressed in metric units.

This means that it will be necessary for you to change some habits of thinking when doing quantitative work. You will need, for instance, to think of centimeters rather than inches. You will probably find that this new way of thinking of measurements is rather easy and that soon you also will have adopted this more universal method of expressing measurements.

The following exercises are provided to give you preliminary experience with the metric system. As you do these exercises you may find that at first you must compare the metric measurements to the English system for the measurements to have meaning for you. Eventually, you should be able to think solely in terms of metric units.

The metric unit of length is the meter (abbreviated m). A meter equals approximately a ten-millionth of the distance from the Equator to the North Pole of the earth. A meter contains 100 centimeters (cm), 1000 millimeters (mm), 1 million microns (μ), 1 billion millimicrons (mμ). A thousand microns make one millimeter. One millimicron is a thousandth of a micron, just as a millimeter is a thousandth of a meter.

Your teacher will provide meter sticks and rulers marked in millimeters. To convert measurements from the metric to the English system, you need to know that 1 meter equals 39.37 inches.

The meter stick is divided into how many centimeters? (1) How many millimeters? (2) How many inches? (3) How many centimeters correspond to one inch? (4) How many millimeters correspond to one inch? (5) How many millimeters correspond to one centimeter? (6)

Use the meter stick to find the length and width of the top of your laboratory table. Record these measurements in your laboratory notebook [in meters (m), centimeters (cm), and millimeters (mm)]. (7)

Using the same units of measurement as for Question 7, use the ruler to find the dimensions of your textbook and laboratory manual. Record these measurements in your notebook. (8)

Greek Fraction Prefixes	Applied to a Meter
$\dfrac{1}{tenth}$ = deci	$\dfrac{1}{tenth}$ meter = 1 decimeter (dm)
$\dfrac{1}{hundredth}$ = centi	$\dfrac{1}{hundredth}$ meter = 1 centimeter (cm)
$\dfrac{1}{thousandth}$ = milli	$\dfrac{1}{thousandth}$ meter = 1 millimeter (mm)
$\dfrac{1}{millionth}$ = micro	$\dfrac{1}{millionth}$ meter = 1 micron (μ, Greek letter "m")
$\dfrac{1}{billionth}$ = millimicro (a thousandth of a millionth)	$\dfrac{1}{billionth}$ meter = 1 millimicron (mμ)

Part B: Using the Metric System for Microscopic Measurements

You can easily calculate the amount of magnification produced by your own microscope. If the ocular lenses magnify an image ten times (10×) and the objective lenses magnify another ten times, then the total magnification of the microscope is 100×. That is, an object viewed in the microscope would appear 100 times larger than it looks to the naked eye at the same distance. Observe the numbers indicating magnification on the ocular and objectives of your microscope and state the total magnification of your microscope with the low-power objective in place. (9) State the total magnification of your microscope with the high-power objective in place. (10)

Although we can thus calculate the total magnification of the microscope, we do not know the size of the object being observed unless we have a reference scale of some kind. In research laboratories a reference scale engraved on a glass disk is placed between the two lenses of the ocular. The scaled disk is called an **optical micrometer**. After proper adjustment, an object can be viewed in the microscope at the same time the ocular micrometer is viewed and the size of the object thus determined directly.

Another way to determine the size of a microscopic object is to compare it with another microscopic object of known size in the same microscope field. For example, a plastic millimeter rule can be placed on the stage across the midline of the microscope field, and an object placed next to it. By this method, the size of the object can be determined directly in millimeters or fractions of a millimeter.

An even more convenient method of approximating the size of an object by means of your microscope is to determine the diameter of your microscope field. Once you know how wide the field is in millimeters or in microns then you can easily estimate the size of an object in that field. For example, if an object extends halfway across your field and the field diameter is 1500 μ, the object is about 750 μ wide. After you have measured the field width once, you can continue to use this method throughout your microscope work.

To measure the diameter of the low-power field, place the 10× (low-power) objective in position. Place a clear plastic millimeter ruler on the stage with its edge across the center of the field. Measure and record the diameter of the field in millimeters, and convert it to microns. (11)

The diameter of the high-power field may be obtained quickly by switching to the high-power objective and looking at the edge of the plastic ruler. Often the scale on the ruler is not sufficiently visible under high power, and therefore the diameter of the high-power field must be calculated by means of ratios. For example, if a high-power objective magnifies 45 times and a low-power objective magnifies 15 times, then the ratio is 3 : 1 and the diameter of the high-power field is ⅓ that of the low-power field. If the low-power field has a diameter of 1500 μ, then the diameter of the high-power field will obviously be 500 μ. State for your own microscope the ratio between the magnification of the high-power objective and the magnification of the low-power

objective. (12) State the diameter of the low-power field in microns as determined in Question 11 above. (13) Calculate the diameter of the high-power field in microns. (14)

Now you can use the information you have obtained on field diameter to calculate the size of a real object. Remove the plastic ruler from the stage and replace it with a wet mount of an algal plant cell. Estimate the dimensions of this cell in microns. For example, if the cell's diameter is about ¼ that of the low-power field, and you have calculated the diameter of the field as 1500 μ, then the diameter of the object is approximately 400 μ (the exact answer is of course 375 μ, but you probably cannot estimate closely enough to justify so accurate a statement).

Now draw one of the algal cells as viewed under low power. Make its size on the paper the same size it appears to you through the microscope. Estimate the largest diameter of the cell by comparing it with field diameter. Measure the largest diameter of your drawing in millimeters. Now compute the magnification of your drawing by using the following equation:

$$\text{Magnification} = \frac{\text{Largest diameter of cell on drawing}}{\text{Largest diameter of cell under microscope}}$$

Obviously both measurements must be in the same units. It will probably be most convenient to make the estimate of the length of the actual cell in microns and then convert it to millimeters, since it is simpler to measure your drawing in millimeters. Record the computed magnifications. (15)

CELLS AS ROBERT HOOKE

FIRST SAW THEM

We will start our observation of cells and their components in much the same way it was first done in 1665 by Robert Hooke, an English naturalist and inventor. You have the same materials and basic equipment that Hooke had —but somewhat improved. The only thing that has not been improved is bottle cork. It has the same structure now as it did the first time it was seen through a microscope.

Cork is obtained from the bark of a cork oak tree native to Spain, North Africa, and Italy. In North America it has been introduced to a limited extent in California. It was used by the Romans as early as 400 B.C. Bottle corks have been made since the 1600's. We know from the way it is used for bottle-stopping, for bobbers on fish lines, for floats on nets, etc., that it is almost impervious to water and other liquids. With this in mind, what function do you think the cork performed when it was a layer of bark covering the trunk and branches of the cork oak tree? (1)

■ **The purpose of this exercise is to look at the cork cells as an introduction to our study of cellular structure and function.**

MATERIALS

Compound microscope	Large bottle cork
New single-edged razor blade	Slide and cover glass
	Pipette

PROCEDURE

Although cork is very easy to cut, freehand thin sections for microscopic examination require careful cutting. *You must be very careful of your fingers.*

Hold the cork firmly in one hand and, with a sharp, single-edged razor blade, slice a thin sliver from the top of the cork by drawing the razor blade obliquely across its surface. With a little practice you can achieve sections thin enough for microscopic examination. The cells will be most easily seen at the thin edge of your diagonal cut. Figure 3-3-1 shows the method of holding the cork and handling the razor blade while cutting the thin freehand sections.

Your teacher may explain to you the use of a nut for cutting sections.

When you have made a properly small and thin section of cork, place the section on a
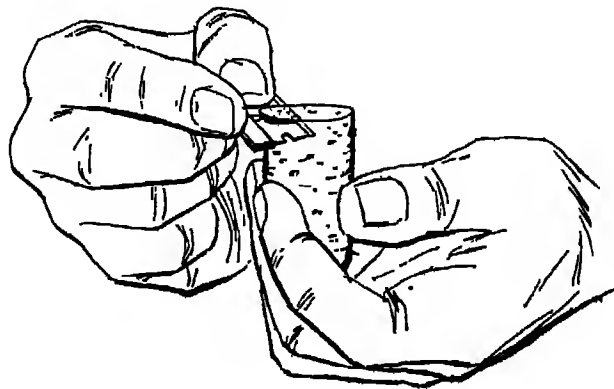
Figure 3-3-1   Cutting freehand cork sections.

clean slide in a small drop of water; add another small drop of water, and carefully cover with a cover glass. Avoid trapping any air bubbles.

Observe the specimen under the low power of the microscope. Look along the thinnest edges of the specimen for the clearest view of cork structure.

Remember you are looking at cells as they were seen for the first time by Robert Hooke. Hooke was the first to use the word cell to describe the units composing the cork.

Draw a small section of the cork to indicate the size and shapes of the cells.

Are all your cork cells the same shape? Why or why not? (2)

What is the actual shape of the cork cells? (3) What structures do you see inside each cell? (4) Do the cells have spaces between them? (5) What part of the cell would keep out the water when cork floats? (6) What do you think would account for the fact that the cork floats in the water? (7) Do you see any evidence that these cells are alive or were once living? (8)

LIVING PLANT CELLS:

Onion Epidermis

Onions are very dead looking when you buy them at the grocery store. In reality an onion is a bulb full of living cells, some of which grow into leaves and roots when the onion bulb is planted (or stored too long where it is damp). Other cells in the onion bulb, less conspicuous in their activity, form a layer of cells covering the bulb scales.

■ The purpose of this exercise is to look at some of these covering cells (epidermal cells) and compare them to Hooke's cork cells.

MATERIALS

Compound microscope	Slide and cover glass
Onion bulbs	Paper toweling
Iodine or methylene blue solution for staining	Needle
	Forceps
	Razor blade
Pipette	

PROCEDURE

Cut the onion bulb into quarters as shown in Figure 3-4-1. You will discover that it separates neatly into layers, called scales. Hold one of the scales so that the concave surface is toward you. Now tear the scale as shown in the drawing.

A transparent, paper-thin layer of epidermis pulls easily from the surface of the bulb scale. It comes off in a sheet, a bit like peeling skin after a bad sunburn. Make a wet mount of the epidermis as follows:

Place a piece of the epidermis in a drop of water on a clean glass slide, so that the outer face (that is, the surface that was outermost on the bulb scale) is up.

If the piece of epidermis is large, use a new, sharp razor blade to cut the epidermis (still in the drop of water) into a piece about 1 cm

square. This will make a clean cut and usually will not wrinkle the specimen. Remove the excess material with forceps. Wrinkles can be removed with a dissecting needle if necessary.

Put on the cover glass and be sure the water comes to its edges but not beyond. Add water at the edge with a pipette, or remove excess water with paper toweling, as necessary.
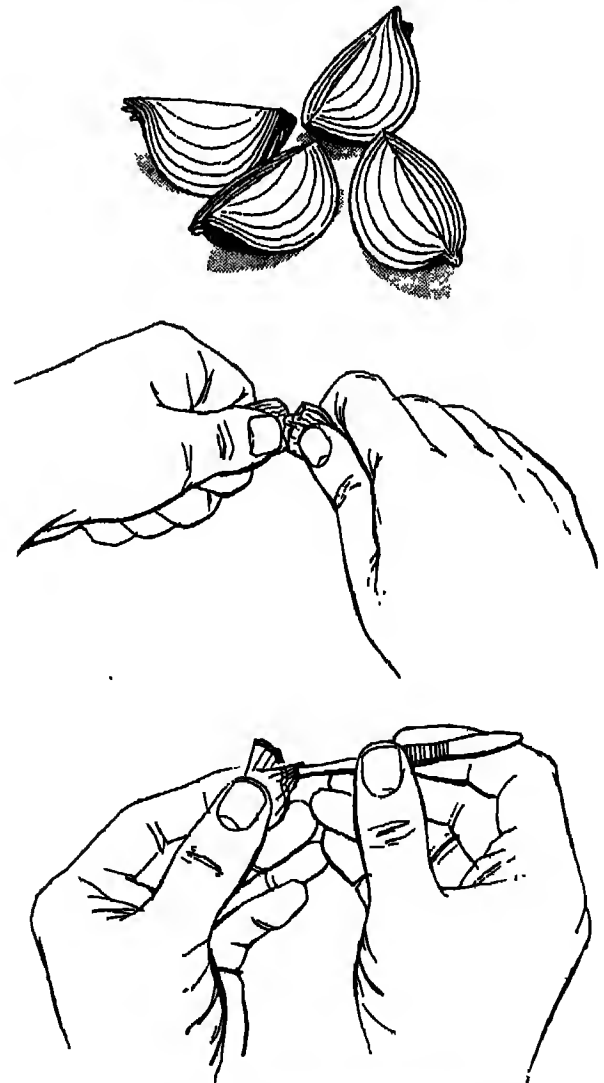
Figure 3-4-1 Preparation of onion epidermis.

Using the low power of the microscope, look at the cells of the epidermis. What is the general shape of these cells? (1) Do they have cell walls? (2) Observe them under high power. What evidence is there that these are living cells? (3)
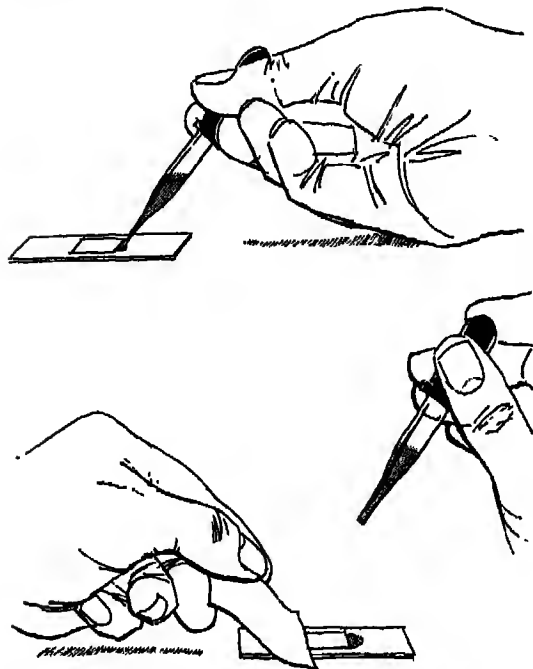
Figure 3-4-2 How to run a liquid under a cover glass.

Now, *take the slide off the stage of the microscope* and run iodine or methylene blue stain under the cover glass in the following way. Place a drop of stain at one edge of the cover glass, then draw it under the cover glass by touching a piece of paper toweling to the opposite edge, as shown in Figure 3-4-2.

Under low power look for a brown- or blue-stained nucleus. What is the shape of the nucleus? (4)

Switch to high power and look at the nucleus again. What is the location of the nucleus in the cell? (5) How can you tell that the cell has depth? (6) Look around the edges of the cell and you will find a fine granular substance that has been lightly stained. This is the cytoplasm. Does the cytoplasm fill the entire cell? (7) Describe what the cytoplasm looks like to you. (8) Is there any difference in appearance between the cytoplasm that has been stained and that seen in the unstained cells above? (9) If there is a difference, how do you account for it? (10)

All living cells contain an abundance of water. Where in the onion cells is most of the water found? (11) Compare the onion cell with the cells of cork. What are the obvious differences? (12) How are they similar? (13)

LIVING PLANT CELLS WITH CHLOROPLASTS

One of the most conspicuous features of our world is green foliage. Although few sights have more aesthetic appeal than the many shades of green in the forest and fields, we usually take this greenness for granted, without pausing to ask why plants are green.

For our first detailed observation of living *green* plant cells, we will use leaves of elodea, a common flowering plant which is widely distributed in fresh-water lakes, ponds, lagoons, and similar bodies of water. We find it frequently in pet shops and variety stores where it is sold for planting in aquaria. (The scientific name of the plant is *Anacharis*, but we shall use the common name, elodea.)

As you observe these living cells in action, keep in mind that the green cells of elodea are very similar in general structure to the green cells of most plants we see growing, and that they perform a similar function: food manufacture in the presence of light (photosynthesis).

■ The purpose of this exercise is to look inside the cells of elodea to discover where the green pigment (chlorophyll) is located. We will be concerned in a later exercise with the characteristics of chlorophyll and its function.

MATERIALS

Elodea
Compound microscope
Finger bowls (4-inch or 10-cm), or other
  containers for plants
Slide and cover glass
Pipette

PROCEDURE

Take one plant from a finger bowl and break off one of the younger leaves near the tip of the branch. Place it bottom side up in a drop of water on a *clean* slide and put on a cover glass.

When you look at this preparation under low power you will see that some cells seem to be packed with small green bodies. These bodies are called **chloroplasts**. What is the difference in function between the cells that contain chloroplasts and those that do not contain chloroplasts? (1)

If the material has been properly prepared you should be able to observe movement of the chloroplasts. As they move in the cell, chloroplasts look like little green beads in a chain—one following the next in rather regular order.

When you have found a cell showing movement of chloroplasts, observe it under high power. What is the shape of a single chloroplast? (2) Where in the cell are the chloroplasts located? (3) Chloroplasts have no means of independent locomotion—they cannot swim or creep around on their own. How, then, can you account for their movement? (4)

Other structures of a living plant cell are present in cells of elodea, but (except for the cell wall) they are obscured by the chloroplasts. With patience and careful observation of many cells you may be able to find a nucleus and may see the cytoplasm—they are there. If you succeed in this, you are developing the power of observation required of all scientists.

If time allows, take a petal of a yellow flower such as nasturtium, macerate it in a drop of water on a slide, use a cover glass, and observe the yellow pigment containing chromoplasts under the microscope.

VARIETIES OF ANIMAL CELLS

Up to the present, you have been studying plant cells. You will now be given the opportunity to observe a variety of animal cells. We shall begin by examining some cells from your own body and then study some cells taken from a frog.

■ The purpose of this exercise is to find out how animal cells resemble plant cells, in what ways they differ from plant cells, and to what degree animal cells differ from each other.

MATERIALS (Parts A and B)

Compound microscope
Toothpick
Methylene blue or iodine stain
Ringer's solution
Four slides and cover glasses
Sperm cells, blood cells, and skin cells from a frog
Pipettes

Part A:
Epithelial Cells Lining the Mouth

You have seen that surfaces of an onion bulb scale are covered by the flat layer of epidermal cells. Parts of animals, too, are covered with an epidermis. This is true of the surface inside your mouth and the external surfaces of your body. In this exercise you are going to look at some of your own epidermal cells.

For obvious reasons it would not be advisable to peel off the epidermis from the lining of your mouth, as we stripped onion epidermis. However, you can, without any harm, gently scrape a few loose epidermal cells from inside your cheek.

PROCEDURE

Place a very small drop of water on a clean slide.

With the broad end of a toothpick, very gently scrape the inside lining of your cheek and deposit a little of the scraping in the drop of water by rolling the toothpick in the water.

Break up the mass by stirring and mincing with the toothpick until there is no longer a detectable mass but only a homogeneous milky drop. Now add a small drop of methylene blue or iodine to the material on your slide. Cover with a cover glass and observe under low power.

Locate a cell. How does the external surface and covering of the cell compare with the walls of plant cells? (1)

You may find some cells creased or piled on top of one another, or some may be broken. Find one or two cells that are clearly visible, well isolated from any others, and quite flat. Center them for viewing under high power, then turn to the high-power objective. The nucleus of each cell should be clearly visible and, if you adjust (usually reduce) the light properly, you may be able to see the cytoplasm. How is the flatness of the cells related to their function? (2) Draw one or two cells. What is the ratio of the diameter of the nucleus to the diameter of the cell? (3)

Part B:
Cells from Parts of the Frog

Most of the cells in an animal are extremely small. Moreover, they are attached to each other so that it is difficult to take them apart. Such cells can usually be observed in sections cut with an instrument called a microtome, which you will read about later. Your teacher may show you some stained sections prepared in this way.

However, there are some animal cells that are relatively large and are normally separate from each other, or that can easily be teased apart with a pair of dissecting needles. Only these cells will be studied in this exercise. Those you will observe are skin cells, blood cells, and sperm cells.

PROCEDURE

Place one clean slide on each of three different pieces of white paper. Write on each piece of paper one of the following titles: BLOOD CELLS, SKIN CELLS, and SPERM CELLS.

Take any one of your three slides and get the proper material from the stock table. Use the following directions for preparing each of the materials.

To the "blood" slide add one *small* drop of blood, a *small* drop of Ringer's solution, a *small* drop of methylene blue, and a cover glass. Observe the cells under the microscope and draw some of these cells under low power and some under high power.

To the "skin" slide add skin cells, a small drop of Ringer's solution, a drop of methylene blue, and a cover glass. Examine them under the microscope and draw two or three of these cells.

To prepare the "sperm cell" slide, the testes were removed from a male frog and cut into very small pieces. They have been standing in a dish of water for a few minutes. Put a small drop of the solution on a microscope slide, cover, and examine and draw as above.

Now answer the following questions about these cell types.

Do the frog blood cells possess a nucleus? (4) Do the skin cells have a nucleus? (5) Describe the shape of the skin cell. (6) Describe the shape of a sperm cell. (7) Describe any cell movement you saw. (8)

LIVING

SINGLE-CELLED ORGANISMS

The microscope reveals not only that plants and animals consist of cells and cell products, but also that there is a whole world of independent tiny plants and animals invisible or just barely visible to the unaided eye. Many of these tiny organisms consist of but a single cell. The microscope enables you to do more than look *at* these organisms; because they are transparent you can observe to some extent what goes on *inside* them. Knowledge of these cells will help us to understand better what goes on inside our own cells.

■ The purpose of this exercise is twofold: first, you will observe the life in pond water to get an idea of the variety of microscopic plants and animals that exists there; then you will select one or two single-celled organisms for a closer study of their life processes.

MATERIALS

Part A
Compound microscope and/or stereoscopic dissecting microscope
Dishes of pond water
Pipette
Slide and cover glass
Methyl cellulose solution

Part B
Pure culture of *Paramecium* or other single-celled animal
Slide and cover glass
Methyl cellulose solution

Part C
Frog intestine
Methyl cellulose solution
Ringer's solution
Slide and cover glass

Part A:

Microscopic Creatures from a Pond

PROCEDURE

You will be provided with a dish containing pond water. In an undisturbed dish, some organisms are found near the surface, others on the bottom, and still others at various depths. With a pipette take a drop from one area in the dish. Put the drop on a clean slide. You need not use a cover glass during this "fishing expedition" if you use low power. *When using high power, always use a cover glass.* The stereoscopic dissecting microscope, if available, is particularly good for this hunt. Later, you will take drops of water from other depths of the dish.

Also note that pond water contains both animals and plants. Some of the plants and animals commonly seen are shown in Figure 3-7-1. List in your notebook the plants and animals you see. You are not expected to memorize the names. They are given so you can look up the organism in a reference book if you wish. If you find organisms not shown in the figures, make a quick sketch so you can identify the animal or plant later if you wish. How do you know which are plant and which are animal cells? (1) You may also find many-celled plants or animals. How do they differ from the single-celled plants and animals? (2)

If you use a compound microscope its field will not cover the entire drop, so do not be surprised if some of the animals and plants swim out of the field and disappear from view. In other words, you see only a small area within the drop. If you want to keep a swimming organism in view, you must continually move the slide. This takes some practice. Also a moving organism may swim out of view in another way: it may swim up or down and thus get out of focus. So you must also continually

Figure 3-7-1

SOME COMMON ORGANISMS

Zygnema
(filament diameter, 12—26μ)

Chlamydomonas
(diameter, 7—8μ)
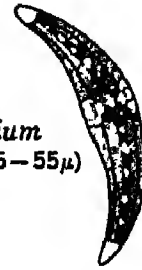
Closterium
(diameter, 46—55μ)

Chaetophora
(filament diameter, 9—12μ)
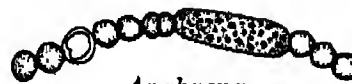
Anabaena
(diameter, 3—11μ)

Micrasterius
(diameter, 100—115μ)

Phacus
(diameter, about 62μ)
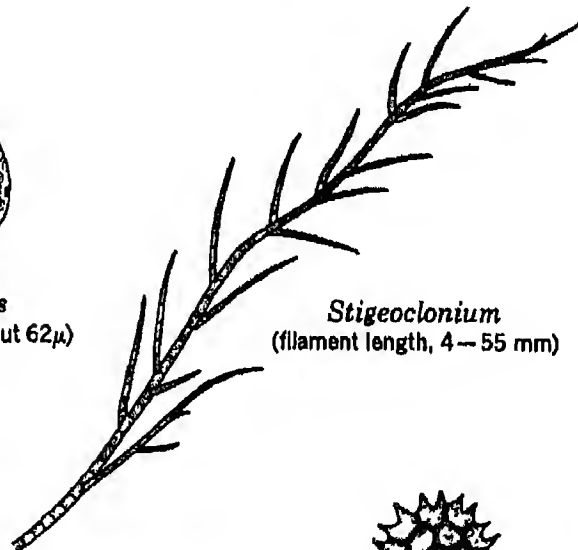
Stigeoclonium
(filament length, 4—55 mm)

Spirogyra
(filament diameter, 36—40μ)

Pediastrum
(diameter, about 240μ)

Pinnularia
(length, 20—80μ)
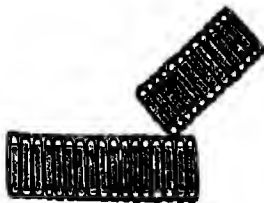
Fragilaria
(length, 20—80μ)

Cosmarium
(diameter about 60μ)

Oscillatoria
(diameter, 5—6μ)
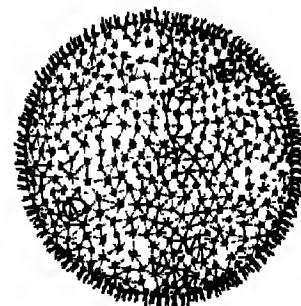
Volvox
(diameter, 650μ;
individual cell diameter, 2—3μ)

FROM A DROP OF POND WATER

Spirostomum
(length, 500—800μ)

Opalina
(length, about 350μ)
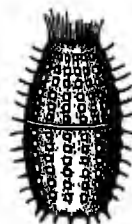
Coleps
(length, 80—110μ)

Stentor
(length, 500—1000μ)
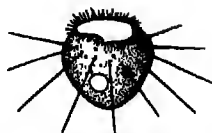
Halteria
(length, 20—40μ)

Chilomonas
(length, 20—40μ)
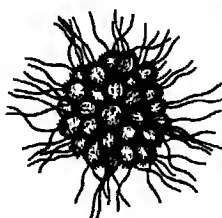
Synura
(diameter, 100—400μ)

Actinophrys
(diameter, 40—50μ)

Blepharisma
(length, 120—180μ)

Colpidium
(length, 80—160μ)
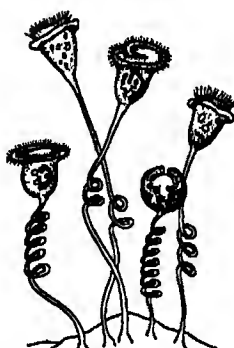
Vorticella
(height, 25—460μ)

Peranema
(length, 20—70μ)

Nyctotherus
(length, 90—185μ)

Euglena
(length, 25—30μ)
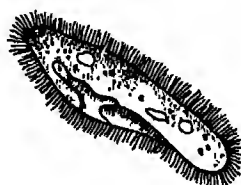
Paramecium
(length, 200—350μ)
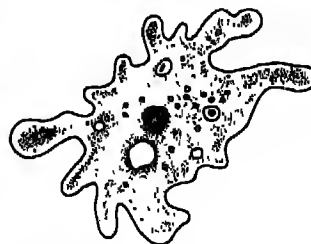
Amoeba
(diameter, about 600μ)

focus the microscope up or down to keep the animal or plant in view. By both moving the slide and also focusing the microscope up or down with the fine adjustment you can keep even a rapidly swimming animal in sight after a little practice.

When you have seen most of the organisms in the drop of water on your slide, wash the slide and cover glass and dry them. Then examine another drop, taken from a different depth as mentioned before. Keep doing this until you are satisfied that you have seen most of the different kinds of organisms in the sample of pond water. Do you get the same organisms from various depths? (3) How many different species did you see? (4)

Should you happen to see anything remarkable, such as an organism reproducing or a vacuole contracting within a cell, make a note of it and call it to the attention of the teacher.

Part B:
Single-celled Animals

What is there about a single-celled animal that you can observe? First, you might find out in what manner and by what means it moves. You might observe how it takes in its food and how it gives off solid wastes and excess water. You could look inside the cell to observe the food vacuoles in which food is digested, and to observe the flow of cytoplasm within the cell.

Common single-celled animals (protozoans) in which these things can be observed include *Amoeba*, *Paramecium*, *Blepharisma*, and *Stentor*.

PROCEDURE

Place a drop of water or methyl cellulose solution containing the organism you are to study on a clean slide and carefully (so as not to crush the organism) cover it with a cover glass. The jellylike methyl cellulose slows down movements of the protozoan so you can see the animal better. Locate a single organism under low power and keep it in the field under

observation *for at least 5 minutes.* The longer you patiently watch, the more you will see. How does the organism move? (5) Do all the single-celled organisms you observe in this exercise move by this same means? (6) Describe the types of movements of the animals and plants you have seen. What happens when the organism runs into something? (7) As the water under the cover glass evaporates, the cover glass will drop closer and closer to the slide. The organism is then caught between the slide and cover glass and can no longer move about. This will be your opportunity to focus on it with high power and to take a good look at what is going on inside the cell. Do you notice any movement of the cytoplasm such as a current or a contraction? (8) If so, describe it. Do you see any chloroplasts? (9) What happens to the organism when the water finally evaporates? (10)

Part C:
Single-celled Animals
from the Frog Intestine

There are single-celled animals that live inside the intestines of larger animals such as the frog. (The human intestine also contains such inhabitants.) To observe the animals from the frog's intestine, a piece of intestine is placed in Ringer's solution, cut open, and macerated (minced and crushed). Drops of the solution can then be transferred to a slide using a pipette and the animals observed. Two single-celled animals generally seen in such preparations are *Opalina* and *Nyctotherus*. How do they resemble the free-living animals seen in Parts A and B? (11) Because they live in the intestine they show certain adaptations to a life inside an animal host. Observe *Opalina* carefully. Is there any special place where food materials can enter the cell? (12) Can you suggest how food enters this animal? (13) Why would one not expect the animal to be free-living? (14) Compare *Nyctotherus* with *Opalina* in relation to the points raised in Questions 12–14. (15)

GENERALIZED CELL STRUCTURE

In the previous laboratory exercises, you have had opportunities to observe plant and animal cells of various kinds, both living and dead.

Through reading your textbook and through your laboratory experiences you now have become acquainted with some of the methods of science. You know that after a scientist has made a number of observations he attempts to make a generalization. That is, he combines his separate observations into a general summary of the phenomenon he has been observing. A generalization is a sort of hypothesis which, in turn, must be tested by further observation or experimentation.

In this exercise you will make generalizations from your laboratory observations in the same manner as a scientist; you are to attempt some generalized drawings of cells. First you will draw a generalized cell, then a generalized plant cell, and finally, a generalized animal cell.

You are to use only those observations derived from your own work on the cell in the laboratory. For this purpose you should review the data you have collected in the laboratory in the previous exercises. You should not, at this time, consult your textbook or any reference book but attempt to utilize only *your observations* in drawing the generalized cells.

■ The purpose of this exercise, now that you have made a number of observations on the cell, is to attempt to summarize your observations in the form of a generalization which will apply to all cells.

MATERIALS

Paper
Pencil
Laboratory notes from Exercises 3-3 through
3-7

PROCEDURE

You have observed during the past few laboratory periods a number of different kinds of cells. You have found, regardless of whether they are plant or animal cells, that they have certain structures in common. Review your records of the exercises on cells and then list those structures *you* have seen which are found both in plant and animal cells. (1)

Using the above list, draw a cell including those parts you have listed and label each part. The generalized cell should be a possible cell shape but not that of any particular specialized cell. The outline should be at least 8 cm long.

Make another list of those structures that you saw *only* in plant cells. (2) Draw, to the same scale as before, a generalized plant cell, labeling all the parts from the generalized cell above plus those that are peculiar to the plant cell.

List those parts of the cell which you saw *only* in animal cells. (3) Sketch and label, in the same way as before, a generalized animal cell.

Now, compare the three drawings you have made. In your drawing of the generalized cell a less experienced biology student should find the kinds of structures he might expect to see generally in cells, but not necessarily in any one cell.

What structures do you see in the plant cell that you do not see in the generalized animal cell? (4) Which of the two cells—generalized animal cell, generalized plant cell—more closely resembles the generalized cell you originally drew? (5)

Prepare a statement in which you describe the major characteristics of (a) the generalized cell, (b) the generalized plant cell, and (c) the generalized animal cell. Suppose you now form, on the basis of your observations, the hypothesis that all plants and animals are made of cells. How would you go about supporting this hypothesis? (6)

Do you suppose you could ever prove beyond a shadow of a doubt that your hypothesis was correct? (7)

TYPES OF MICROSCOPES

Most cells, whether of animals or plants, are too small to be seen by the unaided eye, as you know by now. Under some conditions the human eye can see exceedingly tiny objects. The dust particles visible in a beam of light shining in a darkened room may be as small as 1 μ in diameter. Similarly, the diameter of some silk strands of spider webs may be as small as 1 μ. Ordinarily, however, particles smaller than 100 μ are not visible under normal light conditions. The vast majority of cells have diameters of less than 100 μ. Because cells are so small and because they are the structural and functional units of living organisms, it is not surprising that microscopes are such an important tool for biologists. Here we have an example of the relationship of instruments to the advancement of scientific information. Advances in instrument design also require development of new techniques in order to use properly the refined instrument.

Our ability, in part, to obtain information depends on the instruments available to us—in this case, microscopes. The better the microscope, the better can be our knowledge of cells. However, a fine eye and a fine brain are also necessary!

■ The purpose of this exercise is to familiarize you with various types of microscopes used by biologists, their particular uses, and their limitations. We will also examine techniques which have been developed for use with microscopic preparations.

MATERIALS (Part A)

Stereoscopic dissecting microscope
Objects for observation under stereoscopic microscope: salt crystals, thumbtacks, insects, flowers or leaves, pins or paper clips
Dissecting needles or forceps

Part A:
The Stereoscopic
Dissecting Microscope

The stereoscopic dissecting microscope. (Figure 3-9-1) has certain advantages for many types of microscopic work. It is more satisfactory for opaque objects, for low-power work, and for dissection or observation of objects too large to be seen as a whole in the compound microscope, even at its lowest power. The usual magnifications of the stereoscopic dis-
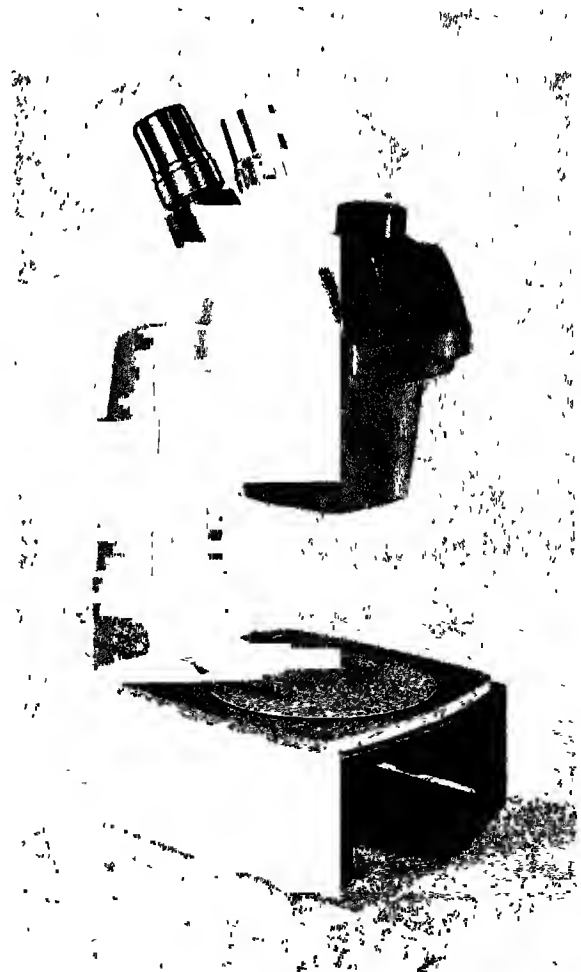
Bausch & Lomb

Figure 3-9-1 Stereoscopic dissecting microscope.

secting microscope vary from 4× to 40×, or even 60×.

This type of microscope is essentially two microscopes in one, because it has two objectives and two oculars. This construction makes the image three-dimensional, as viewed with both eyes. Usually the object is seen by reflected light, that is, you look *at* the object as you might look at a book in your hand with the light reflected from its surface. You have already noticed (Exercise 3-1) that in the compound microscope objects are viewed by **transmitted light**, that is, by the light coming from behind and through the object, as with a stained-glass window.

PROCEDURE

When taking the microscope from its case, carry it in a vertical position by its arm. Place the microscope on the table so that the upper surface of the stage is well lighted.

It may be necessary to adjust the distance between the oculars so that you can comfortably see with both eyes at the same time. The oculars are moved simply by pushing them together or pulling them apart.

Place the object to be viewed (newspaper print, salt crystals, insect, or flower) on the stage of the microscope, approximately in the center.

With the focusing knobs move the objectives down as far as they will go.

Look through the oculars and raise the barrel slowly until the object comes into sharp focus. It may be necessary to readjust the light and the focus. On most binocular microscopes one or both oculars may be adjusted to compensate for differences in focus of your eyes.

While looking into the microscope, move the object being examined to the right and to the left. In what directions does the image move? (1) Move the object away from you and then toward you. Does the image move in the same direction as the object is moved? (2)

Change the objectives to the next higher power and if necessary adjust the light and focus. How does the apparent size of the image compare with that seen under the lower power? (3) Has the size of the field changed in this higher power? (4)

Examine other objects provided. You may wish to compare the appearance of some small transparent object under the stereoscopic dissecting microscope and then under the compound microscope. It is interesting to try dissection or manipulation of specimens with dissecting needles or forceps. Practice manipulating salt crystals, thumbtacks, or other objects. You might then graduate to microdissection of an insect or a flower or another specimen, as a special project. Microdissections are often best done under water by pinning the specimen out in a small wax-bottomed dish full of water or Ringer's solution.

Part B:
The Research Compound Microscope and Preparation of Specimens

As in the low-power stereoscopic microscope you have just used, the more expensive compound microscopes are binocular, but have only single objectives, so the image is not stereoscopic. You are not likely to see one of these microscopes since they each cost hundreds or even thousands of dollars. A typical research microscope such as this is shown in Figure 3-9-2.
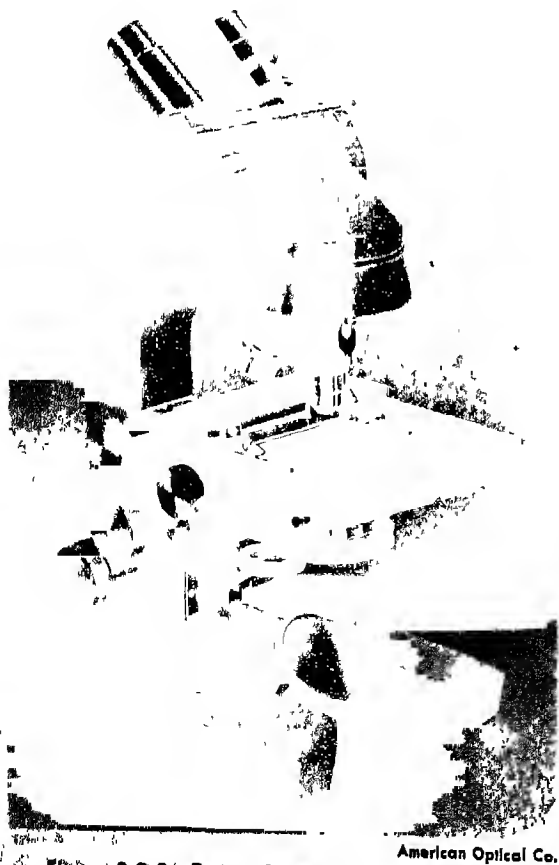
Figure 3-9-2 Research compound microscope.
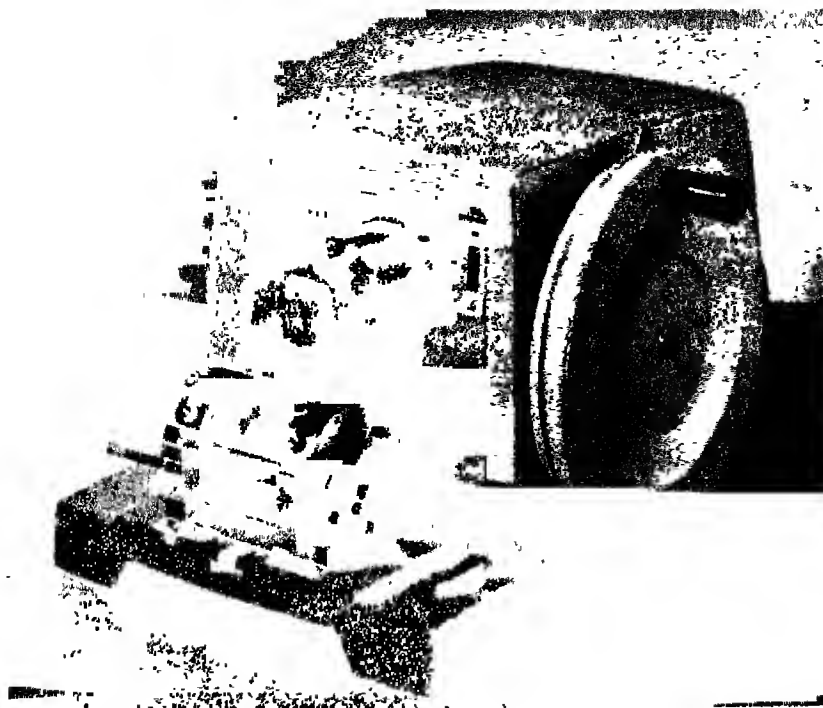
Figure 3-9-3   Rotary microtome.

Because the compound microscope utilizes transmitted light, the objects to be examined must be thin enough to transmit most of the light through the specimen to the eye. Also, because of the dimensions of cells and their internal structures, it is difficult to see even two layers of cells without confusion. Consequently it is necessary to slice the tissues into thin sheets—even thinner than the thinnest sections Robert Hooke was able to cut from cork with a sharp razor. Most prepared sections are cut at thicknesses of 5 to 15 μ. How many sections could be cut from a cell 60 μ in depth? (5)

The instrument used to make these thin sections is called a microtome. It was invented early in the nineteenth century, and works on the same principle as the meat slicer in a meat market.

There are many types of modern microtomes. The type used most commonly is the rotary microtome shown in the photograph (Figure 3-9-3). You can invent simple but effective hand microtomes for your own use, with a bit of ingenuity.

In order to prepare an organism for sectioning, the specimen is first placed in a fixing solution that kills and preserves the tissues. The fixed specimen is then embedded in a block of wax (paraffin) and attached to an arm which is moved up and down by rotating a large wheel. As the arm moves down, the knife cuts a thin slice (or section) from the edge of the block of wax. As the arm moves up again, clockwork advances the block toward the knife a set distance, usually 5 to 15 μ. Thus each turn of the wheel cuts a thin section of wax containing a thin section of the embedded specimen. The thin sheets of wax are then transferred to a glass slide and stuck fast by adhesive. They must, of course, lie absolutely flat! The wax used is dissolved away, and the tissue section on the slide may then be stained. After that procedure, a drop of dissolved plastic is added and the section is covered with a cover glass. (No air bubbles allowed!) When the plastic has dried and the cover glass is held firm, the slide is ready for examination under the microscope.

These accounts of complex and very ingenious machines should make it clear to you that the microscope could not be improved over and over again without bringing about needs for advances in many other techniques: those of slicing the tissues, of preserving them in chemical fixatives, of embedding them in suitable plastics, of staining them with dyes that would stain certain cell structures and not others, and of mounting the sections permanently under glass for examination. This is similar to the evolution of the giraffe's legs, which could not become longer and longer unless the animal at the same time acquired a longer and longer neck, since otherwise it

would not be able to drink from the waterhole.

The history of scientific advancement illustrates not only how the invention of an instrument like the microscope opens previously unsuspected possibilities of exploration, but also how the new discoveries lead to new concepts and theories. Furthermore, it demonstrates how one novel instrument leads to new technical requirements that stimulate the invention of accessory instruments and techniques. These instruments and techniques may, in turn, lead to an improvement in the original instrument.

Part C:
The Phase-Contrast
Microscope

One of the most valuable new developments of the compound microscope is the phase-contrast microscope. This complicated instru-

Figure 3-9-4 Electron microscope.

ment, with optical properties too complex to explain here, gets its name from the fact that various parts of the *living* cell stand out in sharp *contrast* because the *phase* of light is changed.

The great value of this microscope lies in the fact that with its use transparent or nearly transparent structures, such as chromosomes and mitochondria, can be seen plainly. To view these structures is difficult, of course, with an ordinary compound microscope. It is possible only if the material is fixed and stained to make the structures visible, and this of course kills the cells. But it is possible to use the phase-contrast microscope to study these structures while they are living. When the phase-contrast microscope is combined with time-lapse motion picture photography, the movements and changes of the cellular structures during cell division and during other complex cellular activities can be seen very dramatically.

The mitochondria that are so numerous in the cytoplasm of all cells do not stain with the customary dyes used by cell biologists, so they remain invisible in most prepared microscope slides of cells and tissues. But the phase-contrast microscope shows them superbly, clustered in various places, often around the nucleus or the spindle of a dividing cell. These structures carry on most of the respiration upon which life depends. Yet they were indeed mysterious until this microscope revealed more about their shapes, sizes, and numbers, and their tremendous activity.

The phase-contrast microscope is quite a recent invention. It was first produced in the Netherlands by Fritz Zernike in 1935. The first biological film of cell activities made by Zernike with this microscope was captured by the Germans during World War II and later was obtained by the American armed forces. This film is still often shown to schools and other groups.

Part D:
The Electron Microscope

Since the resolving power of the light microscope (compound microscope) is limited by the nature of light itself, it follows that the only means of increasing its resolving power would be to employ shorter wavelengths than those of visible light. This is the principle of the electron microscope (Figure 3-9-4). Elec-

trons, rather than visible light, are used to form an image.
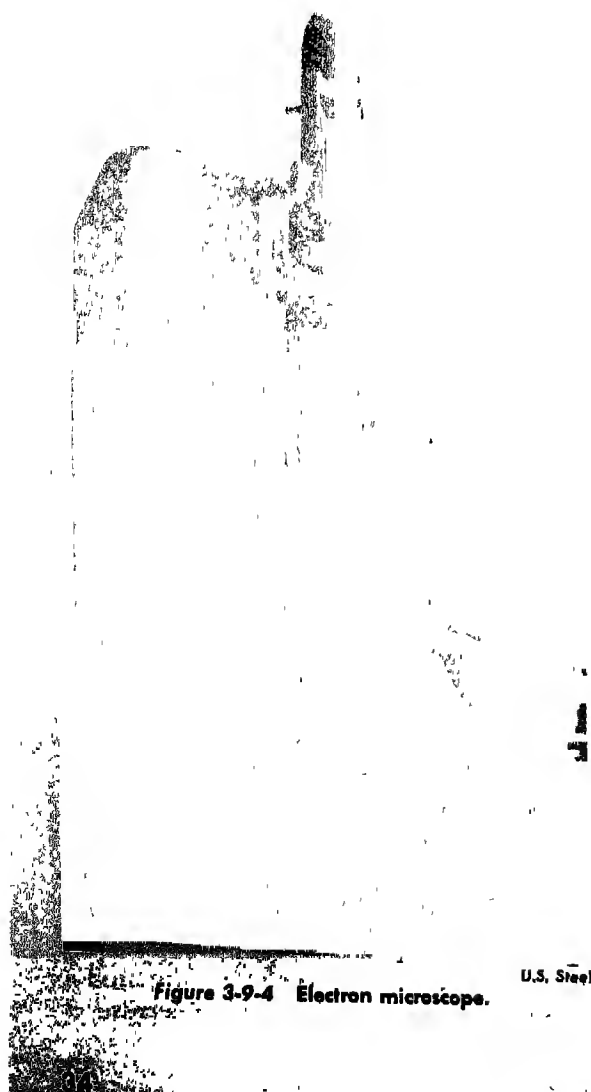
Streams of electrons are given off from a heated wire placed in a cylindrical column from which most of the air has been removed. These streams of electrons have the properties of waves, with a wavelength less than 0.01 mμ. Of course we cannot see these electrons, but a screen can be coated with minerals that glow when struck by electrons. Thus we can see, though indirectly, the image formed by the beam of electrons. This is the principle of a television picture tube.

It has been found that strong magnets (electromagnets) can be used to focus an electron beam, because these electrons are charged particles (see Chapter 5 of the textbook). Magnets in the electron microscope are used in much the same way as glass lenses are used to focus visible light in the light microscope.

Employing this idea, two German scientists in 1932 constructed a microscope that used a beam of electrons rather than visible light. The instrument was named the electron microscope. Gradual improvements were made, and by 1950 the electron microscope had become an invaluable tool for the scientists in the study of cells.

The electron microscope is still being improved. Theoretically, it should be capable of a resolving power 1000 times that of the compound microscope and sufficient to see individual atoms. It is far from this degree of perfection now. But already it is routinely giving resolutions 100 times that of the best compound microscope. Today, a whole new world of the interior structure of the cell is being revealed.

In Figure 3-9-5 you can compare the optical systems of the light microscope and the electron microscope.

You will recall that with the optical microscope you determined the magnification by multiplying the magnification of the objective by the magnification of the ocular. The same procedure applies to the electron microscope. For example, if the initial magnification by the objective is 100 × and if the projector coil (equivalent to the ocular of the light microscope) can magnify 200 ×, what would be the total magnification? (6) In more recent instruments a wide range of magnifications can be reached by introducing an intermediate lens. Direct magnification as high as 100,000 × may thus be obtained, and the **photomicrographs** (photographs that are taken through the mi-

croscope) may be enlarged to 1,000,000 × or more.

This seems to be an ideal instrument, doesn't it? Nevertheless, its usefulness is reduced by a number of technical difficulties. One limitation is due to the low power of penetration of the electrons through solid material. The specimen to be examined must be very thin. It is generally deposited on an extremely fine layer of collodion (7.5 to 15 mμ in thickness). The collodion film serves as an object-holder for the specimen and is supported by a fine metal grid.

If the thickness of the specimen exceeds 500 mμ (or 0.5 μ) it appears almost totally opaque. Would it be possible to view an entire cell of average size with the electron microscope? (7)

Another limitation comes from the fact that since the specimen must be placed in a vacuum, it must be dehydrated. This makes the study of cells in the living state impossible with this instrument. Great improvements that have recently been made in preparation techniques are helping to decrease these limitations to some degree.

Because of their uniform thinness, biological materials such as thin membranes, filaments, or large molecules (having a diameter of 10 mμ or less) possess a very low power to disperse electrons. A shadow technique has been devised to overcome these difficulties. The technique consists of placing a specimen in a vacuum chamber and then vaporizing a heavy metal such as chromium within the chamber. The chromium molecules adhere to the specimen in such a way as to give the image of the specimen in the photomicrograph a three-dimensional effect.

Part E:
Magnification
and Resolving Power
in Different Microscopes

Now that we have looked at the differences between these instruments that so powerfully extend human vision, we may sharpen our idea of their usefulness and their respective limitations by examining the same piece of living tissue when seen through each of them.

LIGHT MICROSCOPE

Observation screen, photographic plate, or eye

Second stage

Projector lenses (ocular)

Intermediate image

First stage

Objective lenses

Specimen

Condenser lenses

Illumination

Light source
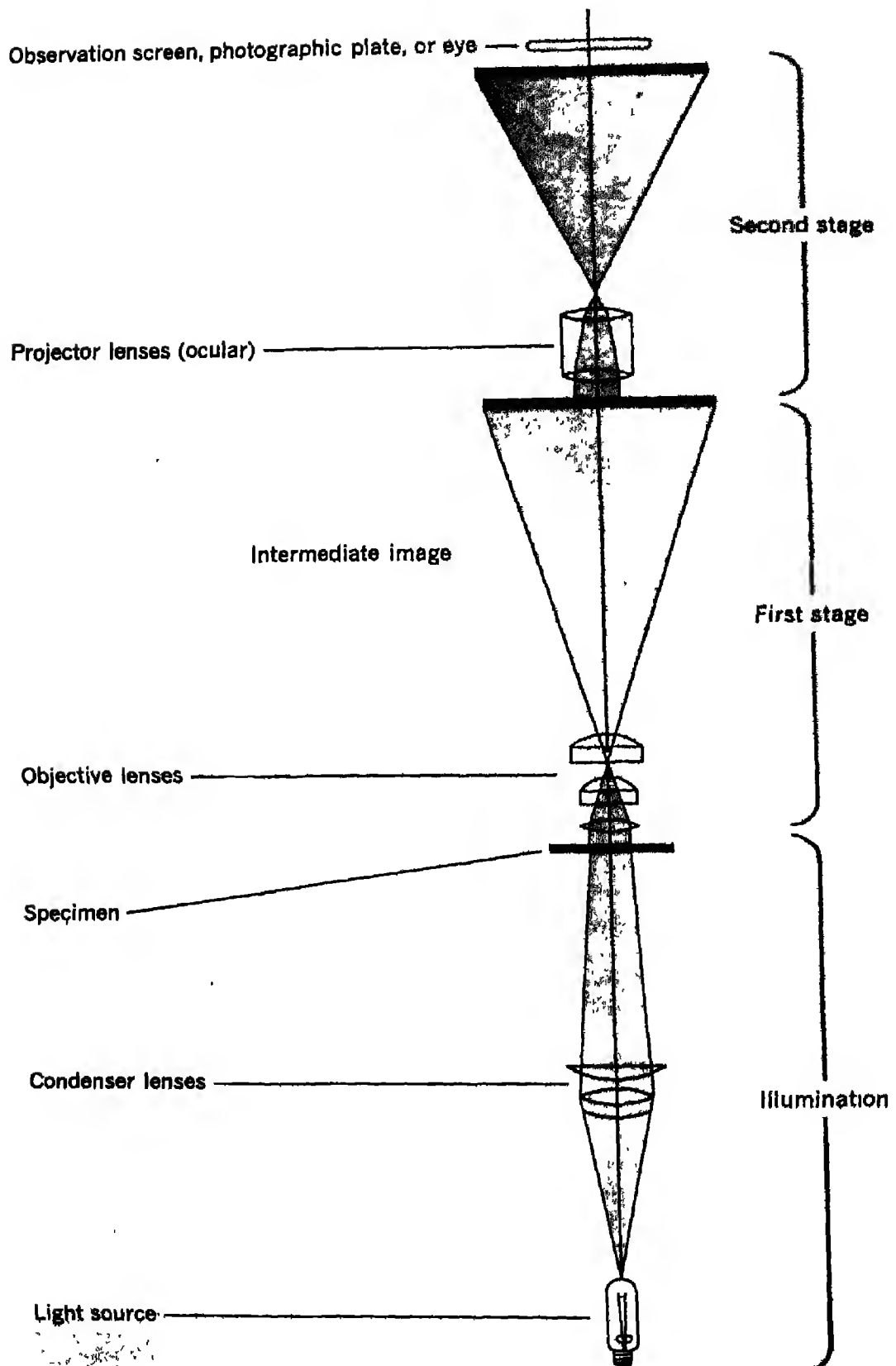
Figure 3-9-5   Schematic drawing of optical systems of

ELECTRON MICROSCOPE

— Observation screen or photographic plate

Second stage

— Magnetic projector (coils)

Intermediate image

First stage

— Magnetic objective

— Specimen

Illumination

— Magnetic condenser

— Electron source
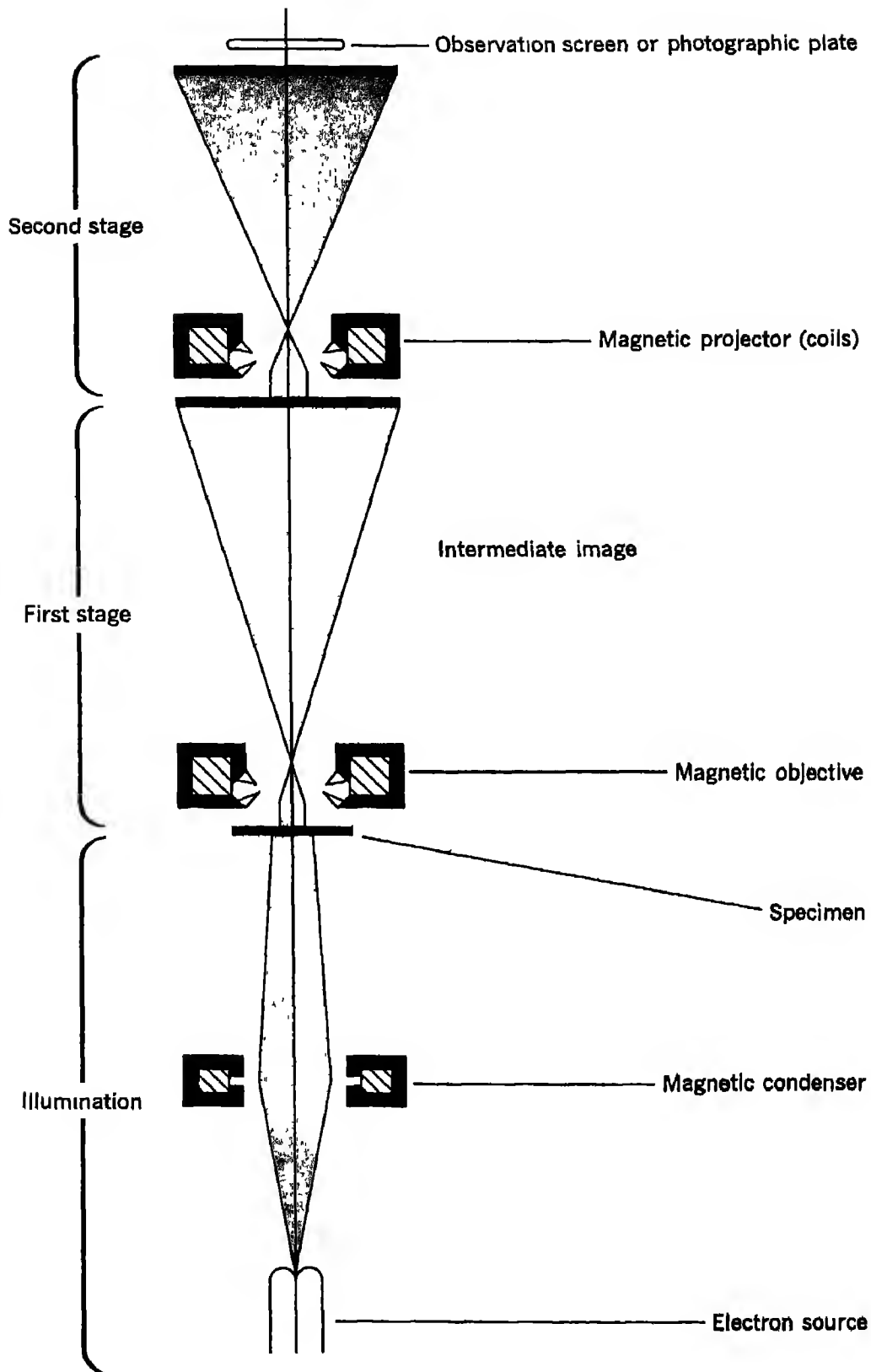
light microscope and electron microscope (inverted).
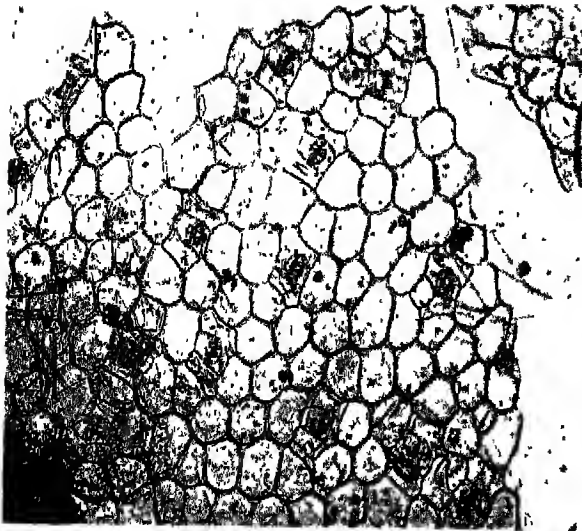
A

B
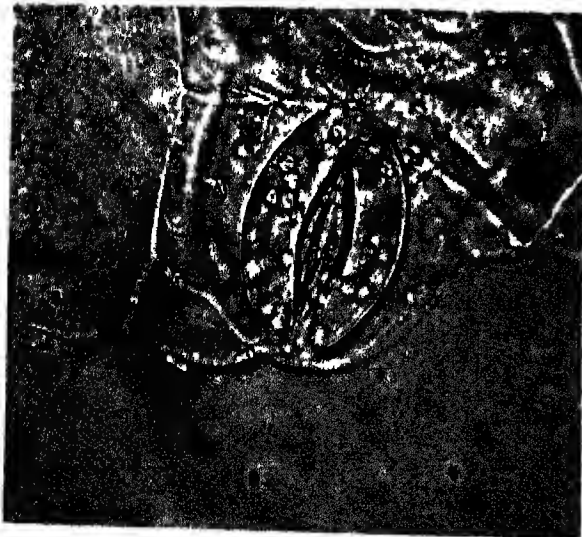
C

D

Photograph A (Figure 3-9-6) shows the lower side of a leaf of a green plant, as it might be seen through a compound microscope at a magnification of 100 ✕. You will see numerous epidermal cells shaped like so many pieces of a jigsaw puzzle; and scattered among them many pores opening into the interior of the leaf. Each pore, or stoma, as it is called, is surrounded by two bean-shaped cells called guard cells.

Photograph B is taken at a magnification of 440✕. To what combination of ocular and objective of your own compound microscope does this correspond? (8) You will see the two guard cells surrounding the stoma much more clearly now. Each one, besides a nucleus, contains numerous small disks. What are these? (9)

Photograph C is taken at a magnification of 1000✕. Although this is beyond the power of your own laboratory microscope, it is not beyond the limits of magnification and resolving power of which research compound light microscopes are capable. Can you see any more detail in the parts of the cell than at 440✕? (10)

Photograph D is also taken at a magnification of 1000✕, but through the phase microscope. Can you observe any structures that are not visible in the preceding photograph? (11) If so, most of them are probably mitochondria.

Photograph E shows two chloroplasts in a similar cell at a magnification of about 10,000✕. It was taken with an electron microscope. The round structures inside the chloroplasts are

Figure 3-9-6
The photographs on these two pages show different magnifications taken through three types of microscopes.

Compound microscope:
(A) 100 ×; (B) 440 ×;
(C) 1000 ×.

Phase-contrast microscope:
(D) 1000 ×.

Electron microscope:
(E) 10,000 ×; (F) 25,000 ×.

(A-D are *Zebrina*,
E-F are *Zea Mays*.)

E
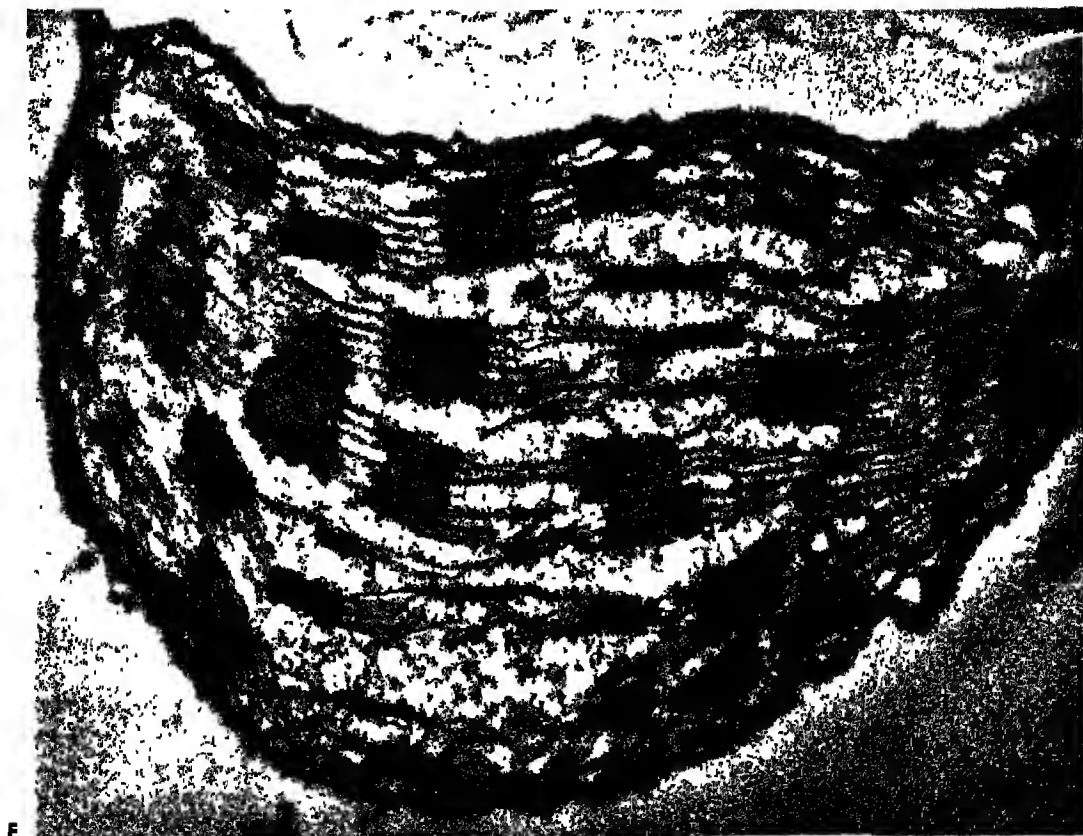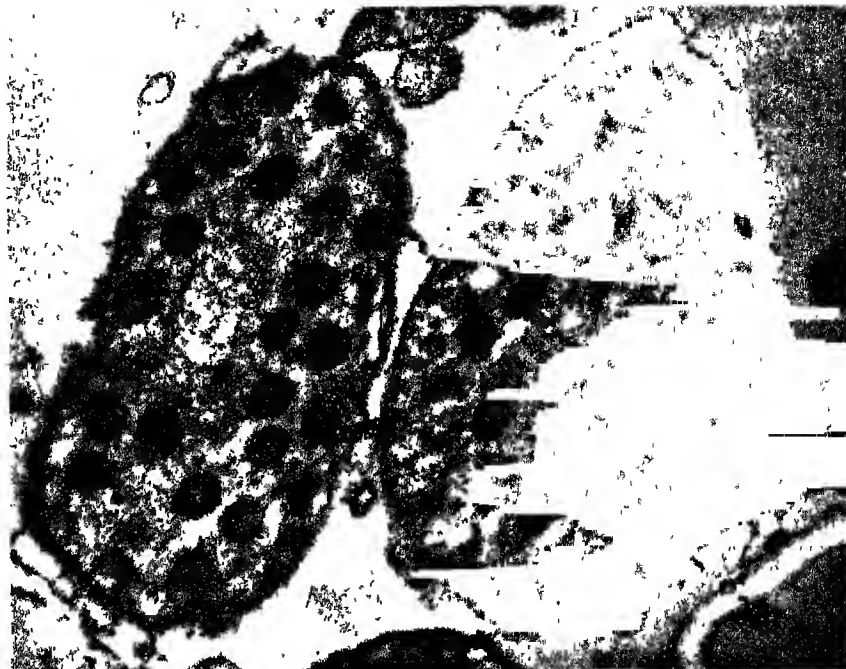
F

called grana. How many can you see in a single chloroplast? (12) Can you make out any internal structure inside a granum? (13)

Photograph F was not taken at a magnification of 100,000×, as you might expect, but only at 25,000 ×. It shows a single chloroplast. De- scribe what you can now see to be the true structure of a granum. (14)

Consider why the grana look different in photographs E and F. Make a stack of eight quarters or other coins all of the same size. Now make a sketch of the pile of coins looking

straight down on it, and beside it make a sketch of the pile of coins as they look when viewed from the side. Does this comparison explain the difference in the appearance of the grana in photographs E and F? (15)

Considering all six photographs, name two pictures in which the increased magnification did not reveal any new indications of fine structure. (16) Also name two photographs in which there was a better *resolution* of fine structure at the higher magnification. (17)

Clearly the biologist, whether a student or a research worker, has at his disposal a very wide range of magnifying devices—from the 3× hand lens to the 1,000,000× electron micro-

scope. Each of the various magnifications finds its usefulness in the study of living organisms. Great resolving power is desirable at all of them. High magnification is unable to reveal more detail without high resolving power. Conversely, high resolving power is useless without sufficient magnification.

To fix in your mind the "sizes of things," complete the chart (Figure 3-9-7) following. For the information you need use your notebook, this laboratory exercise, or any other reference you may wish. Indicate the range of usefulness of the different microscopes listed in the chart in the same way as the range of usefulness is shown for the unaided eye.
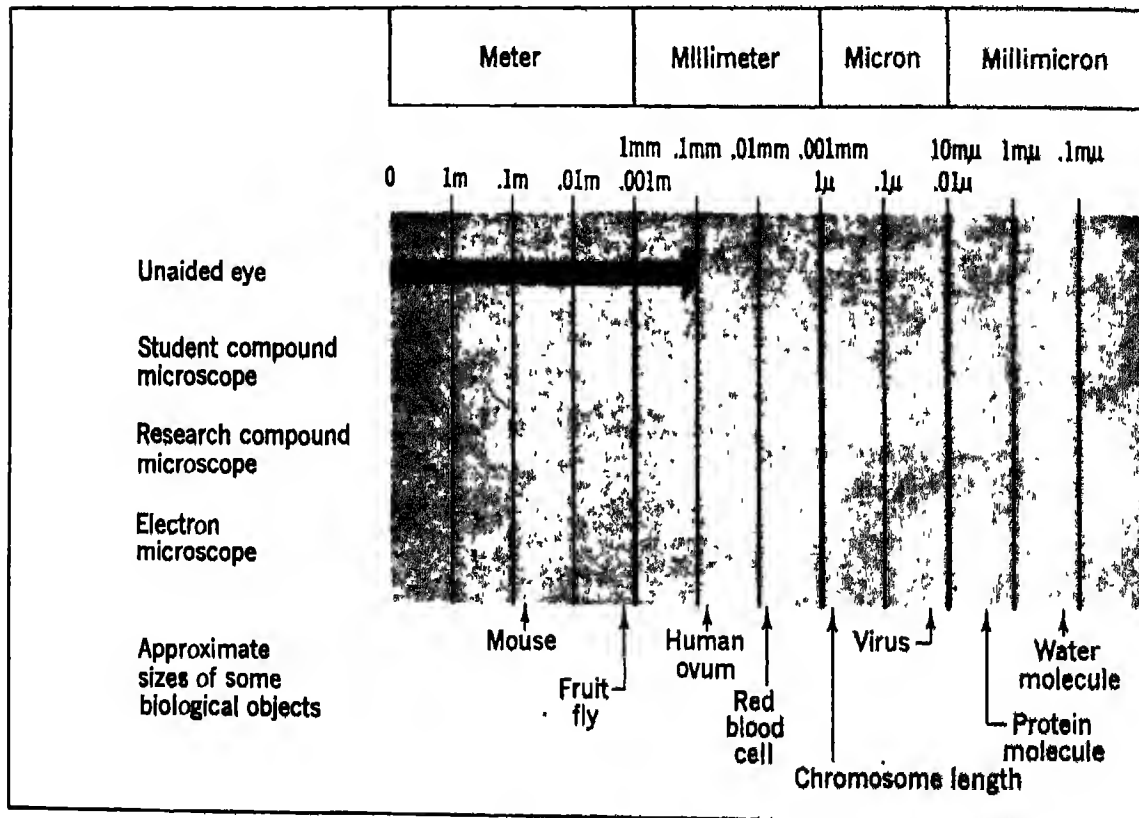
Figure 3-9-7 Sizes of things.

SOME BASIC FUNCTIONS

Early experiments in biology demonstrated that animals use oxygen from the air and that plants produce oxygen. The following exercise is intended to demonstrate the ways in which animals and plants depend on each other in their utilization of certain substances from the air. The experiments are somewhat similar to those of Priestley and of other scientists and may help to illustrate the historical work about which you have read in your textbook.

■ The purpose of this exercise is to show the relationship between plants and animals and air in an experiment similar to that performed by Priestley.

MATERIALS

Four 100-ml beakers

Four 15 mm x 125 mm test tubes or shell vials

Two healthy houseflies, cockroaches, or other insects

Two green leafy plant shoots (such as a large blade of grass) about 7 cm long

Filter paper or paper toweling

Sugar solution

Notebook paper

Two pellets of sodium hydroxide (NaOH)

Spoon, porcelain

Forceps

Millimeter ruler

Wax pencil

100-ml graduated cylinder

PROCEDURE

We shall begin with the preparation of four test tubes used in the four parts of the experiment. While you are reading the instructions, look also at Figure 4-1-1. The tubes are prepared as follows.

Measure exactly 30 ml of water by means of a graduated cylinder and pour the water into a 100-ml beaker. Repeat this procedure for the other three beakers.

Into Tube 1 place a small piece of filter paper moistened with sugar solution. Handle the filter paper with forceps and attempt to place it near the bottom of the tube without touching the sides of the tube any more than necessary.

Prepare a small wad of notebook paper to form a fairly tight plug which you can push about halfway down into the test tube. Test the plug to make sure it fits just tight enough to stay in place and then remove it and insert one insect into the test tube. Be extremely careful of the insect; if you injure it the experiment will not give valid results. As soon as you have placed the insect in the tube, insert the paper plug and push it about halfway down into the tube to keep the insect in the tube.

Invert the test tube and place the open end into one of the beakers filled with 30 ml of water. Lean the tube against the side of the beaker, as shown in Figure 4-1-1.

Into Tube 2 insert a piece of filter paper soaked in sugar solution and a paper plug, as you did with Tube 1 above. Insert a leafy plant

Filter-paper fragment
Vial or test tube
Insect
Paper plug
Leafy shoot
Water
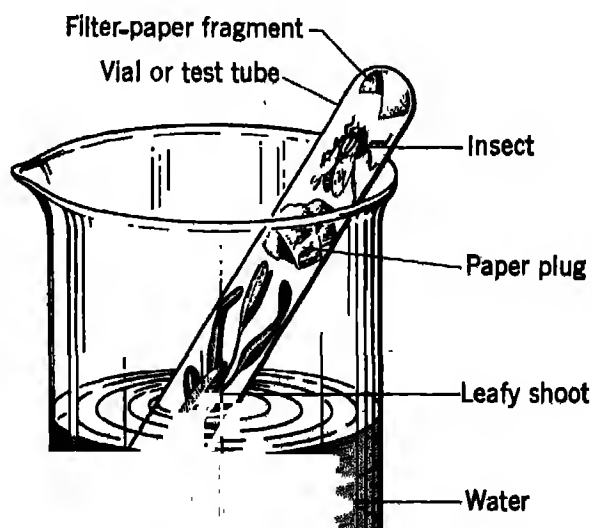
Figure 4-1-1   Preparation of Tube 3.

shoot into the tube in such a way that the tip touches the plug and the cut end is approximately flush with the opening of the tube. Invert the tube and place it into another beaker containing 30 ml of water, as with Tube 1 above. Be sure the cut end of the shoot is in the water.

In Tube 3 place both an insect and a leafy plant shoot. The method is a combination of those used for Tubes 1 and 2 above. First place a piece of filter paper soaked in sugar water in the bottom of a test tube. Place an insect in the tube and quickly insert the plug. Then place the green leafy shoot into the tube until the tip touches the plug and the cut end is approximately flush with the open end of the tube. Invert the whole system in one of the beakers containing 30 ml of water.

Make Tube 4 as you made Tube 1 above, but include only filter paper soaked in sugar solution and a plug. At this stage in your scientific career you should already be able to explain the purpose of making Tube 4. What function does Tube 4 perform in the experiment? (1)

Two kinds of observations are to be made. It is desirable to determine the approximate time of survival of the insects in Tubes 1 and 3. It is likely that the insects will die within a few hours, but they may live longer. Your teacher may assign individual students or teams to make observations at various times during the day to observe how long the insects in these two tubes survive. On the basis of these records, explain any differences observed in the length of lives of the insects in Tubes 1 and 3. (2)

You can also measure changes in the volume of gas in each tube, and determine what substances are used and produced by the organism in each tube in the following way.

Make the first measurement with your ruler just after you complete setting up the experiment. Mark the level of the air column on the outside of the test tube with a wax pencil. Record for each of the four tubes the length (directly related to volume) of the air column in the tube in millimeters. (3)

Observe the tubes daily and record your observations. When the insect in Tube 1 dies the observation period is considered to be finished. Conclude the experiment as follows:

Measure the length of the column of gas in Tube 1. State the amount of increase or decrease as a percentage of the volume you measured at the beginning of the experiment. (4) How could you determine what gas has been produced by the insect? (5)

Add to the water in the beaker two pellets of sodium hydroxide. (DANGER: *NaOH will burn the skin or clothing. Do not touch the pellets*, but use a spoon, forceps, or some other implement to pick them up.) Mix thoroughly with a stirring rod so the NaOH mixes with the water in the test tube, but do not remove the test tube from the water. NaOH solution is a much better solvent for carbon dioxide than is water. With this piece of information in mind, explain the change in water level in the test tube, which will probably occur as the NaOH dissolves. (6) Summarize your conclusions regarding Tube 1. (7)

Make similar observations of Tubes 2, 3, and 4, following the same procedure as for Tube 1, including addition of NaOH. In an organized way, state your conclusions concerning each of these tubes. (8, 9, 10) What factors might explain any observed changes in volume in Tube 4? (11) How would changes in volume in Tube 4 affect your interpretation of the changes in Tubes 1, 2, and 3? (12)

In a short paragraph, give an organized summary of the conclusions from the observations made in this exercise. (13)

THE ANALYSIS OF WATER

There are two ways in which we can deal with chemical substances. If we wish to find their composition, we can take them apart to determine their individual components This taking apart is referred to as analysis. The opposite of analysis would be putting together. The process of putting together is termed synthesis.

The most abundant compound in living material making up 80% of the cytoplasm, is water, the role of which is covered extensively in Chapter 5 of the textbook. Water is essential for the two chemical reactions most fundamental to life, hydrolysis and most biological oxidations. In addition, it serves as a temperature stabilizer and as an excellent solvent. In this exercise we will deal with the analysis of water and subsequently its synthesis.

Water is both analyzed and synthesized in the chemistry of living organisms. Part of the process of photosynthesis involves the breaking up of water into its two elements, hydrogen and oxygen. The source of the energy for this splitting process is light. The process of splitting (analyzing) by means of light is called photolysis. The following equation summarizes the photolysis of water using light energy:

$$2H_2O + \text{light energy} \longrightarrow 2H_2 + O_2$$

(chlorophyll
for absorption
of light energy)
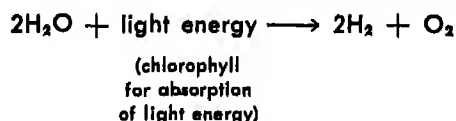
A simple test for hydrogen is its explosive nature when ignited by a spark or burning splint in the presence of air. Oxygen is the gas which supports combustion and can be tested for easily. When a glowing splint is immersed in oxygen, the splint bursts into flame.

■ The purpose of this demonstration is to show the chemical composition of water as determined by analysis and subsequently to show the synthesis of water and to determine the energy relationships between these two processes.

MATERIALS

Hoffman electrolysis apparatus
6- to 10-volt direct current source
Concentrated sulfuric acid (H_2SO_4)
Wood splinters
Two test tubes
Rubber tubing and pinch clamps (if the Hoffman apparatus is not fitted with petcocks)
Wires for connecting power source to Hoffman apparatus
Matches

PROCEDURE

The procedures to be followed in this demonstration are as follows:

Open the tube petcocks or remove pinch clamps from the rubber tubing at the end of the apparatus. Fill the electrolysis apparatus with water up to the *bottom* of the reservoir.

Close the tube petcocks or replace the pinch clamps.

Connect the wires from the platinum electrodes of the apparatus to the direct current power source. Add a few drops of concentrated sulfuric acid to the water, taking care not to get acid on your skin or clothing. What changes are noticed upon the addition of the sulfuric acid? (1)

Continue the experiment and observe the collection of gas in the upper parts of the tubes. Is the amount of gas the same in each tube? (2) After sufficient gas has been collected to permit measurement, record the amount of gas in each tube. What is the ratio between the amounts of gas in the two tubes? (3)

Does the splitting of water into its two components require energy? (4) What is the source of energy in this experiment? (5) Do the separate gases in the tubes of the electrolysis apparatus contain more or less potential chemical energy than they did when they were combined as water molecules? (6)

Invert a test tube over the end of the tube of the apparatus containing hydrogen. Release the gas into the inverted test tube. Cover the mouth of the test tube with your thumb and remove it from the vicinity of the apparatus. Tilt the tube to about 45° and hold a lighted splint near the mouth of the test tube. What is observed? (7) What does this signify? (8) Examine the mouth of the test tube closely. What is observed around the mouth? (9) How do you account for this? (10) Did this reaction to the lighted splint require energy or release energy? (11) Was the reaction observed at the mouth of the tube an example of analysis or synthesis? (12)

Collect the gas from the other tube in the same manner as the hydrogen was collected. Insert a glowing splint in this test tube and observe the results. What happens when the glowing splint is introduced into the test tube? (13) How does this reaction support the assumption that oxygen was in the tube? (14) Was energy released in this reaction? (15) In what form? (16)

Refer back to the formula for water. Which of the two tubes contained the hydrogen, that with the greater or that with the lesser amount of gas? (17) Which contained the oxygen? (18) What can possibly account for your answers to 16 and 17? (19)

Referring back to the formula for the photolysis of water given at the beginning of this exercise, describe how this demonstration parallels the splitting of the water molecule by photolysis. (20) You already know from reading your text that oxygen is released in photosynthesis. Is there any indication that hydrogen is released in the process of photosynthesis? (21) What becomes of the hydrogen released in the plant during photolysis? (22)

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DIFFUSION

THROUGH A MEMBRANE

In this exercise you will observe certain characteristics of membranes. Membranes, whether cell membranes or the cellophane dialysis membranes that we shall use, allow the passage of certain molecules and prevent passage of others. The cellophane dialysis membrane that we will use permits slow passage of water and other small molecules through itself but does not allow other larger molecules to pass through.

This slow passage of water molecules is a type of diffusion, initiated by random Brownian movement. Diffusion is discussed in your textbook under the heading, Water, in Chapter 5 and also in Chapter 6.

■ The purpose of our study of diffusion through a nonliving membrane is to provide a background for the understanding both of diffusion in living cells and of the action of an enzyme and its effect on diffusion.

MATERIALS (Parts A, B, and C)

80% Glucose solution	Two gas collection bottles or small instant coffee jars
Soluble starch solution	Three test tubes
Iodine solution	Test-tube holder
Benedict's solution	1-ml pipette
Bunsen burner	Ring stand
Clinitest tablets or Tes-Tape	Clamp
	Meter stick
String and rubber bands	Thermometer
	Graduated cylinder
1-cm (⅜-inch) cellophane dialysis tubing; three pieces each, 20 cm long	Wax pencil
	Pipette (medicine dropper)

Part A:

Diffusion of Starch and Glucose

PROCEDURE

Tie a knot very tightly at a point about 1 cm from one end of a piece of cellophane dialysis tubing and fill the tube to within 5 cm of the top with soluble starch solution as shown in Figure 6-1-1A.

Add about 20 drops of glucose solution to the cellophane tube.

Tie the top of the cellophane tube tightly with string and rinse it under running water to remove any glucose or starch that may have spilled on the outside.

Place the filled cellophane tube in a container of water. (See B of the drawing.) Add to this water 5 ml iodine for each 50 ml of water in the container. The development of a blue color is a test for starch.

After 15 minutes, test the external solution for glucose by adding a drop of the liquid from the bottom of the container to a fragment of a Clinitest tablet, or dip a piece of Tes-Tape in the liquid. A yellow or green color indicates the presence of glucose.

Observe the contents of the cellophane tube and the liquid surrounding it. Record the changes and the times at which they were noted. (1) On the basis of the chemical tests for starch and for glucose, what must have happened to the iodine in your experimental set-up? (2) To the starch? (3) What happened to the glucose? (4) What materials have diffused through the membrane? (5) What substances have not diffused through the membrane? (6)

Molecules of iodine are smaller than molecules of glucose; a molecule of starch is made up of many molecules of glucose bonded together. Suggest a hypothesis to account for the failure of starch to diffuse through the mem-
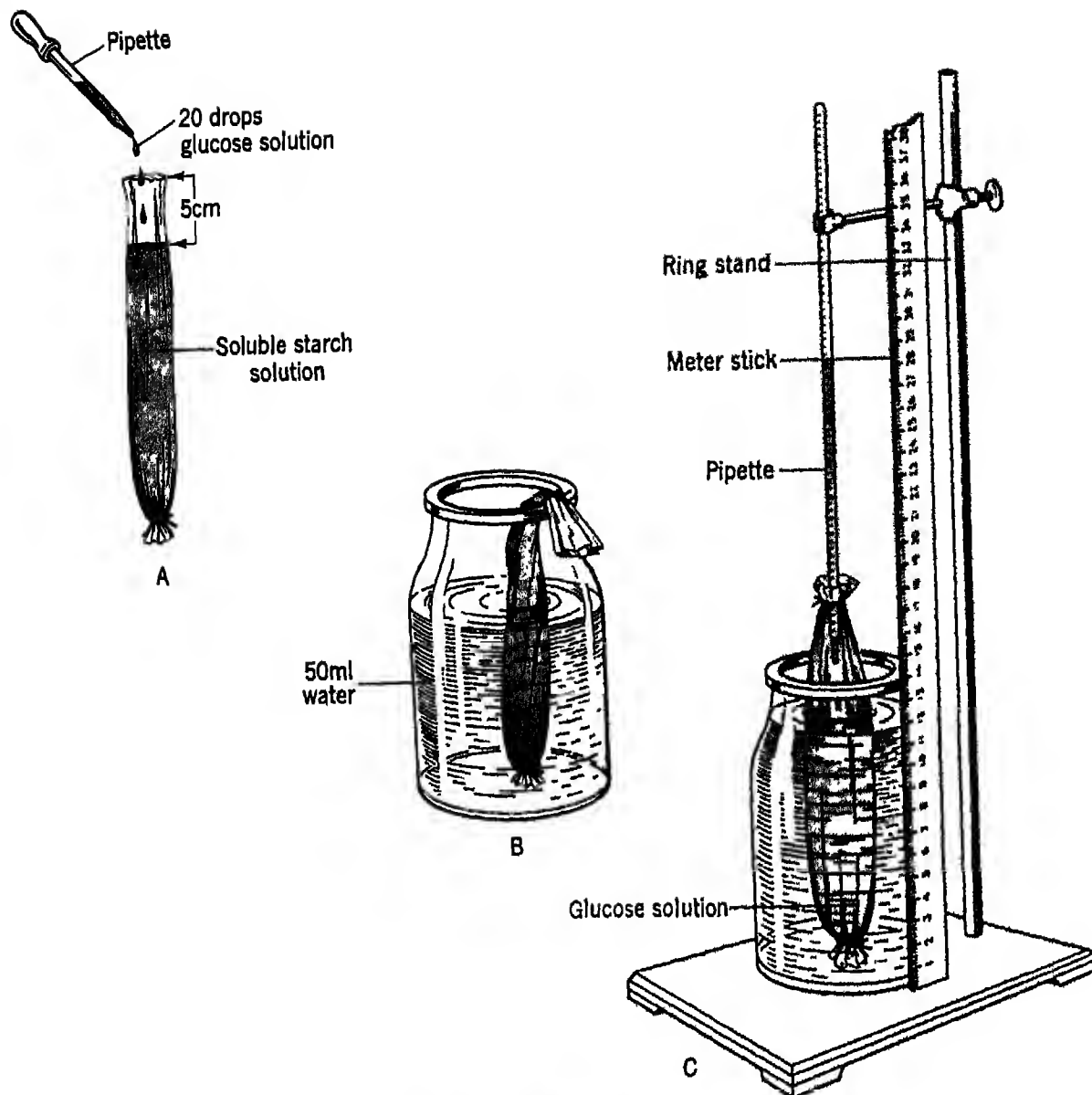
Figure 6-1-1 Diffusion apparatus.

brane. (7) What did you imagine the structure of the cellophane tube to be when you made this hypothesis? (8)

If a membrane permits some substances to pass through and prevents or slows down the passage of other materials, it is said to be semi-permeable. Can materials diffuse through a semipermeable membrane in both directions at the same time? (9) What materials diffused in opposite directions in this experiment? (10) As you do these experiments with the dialysis tubing, remember to relate what you are seeing to the living cell. Remember that the living cell is never in equilibrium with its environment in terms of materials across its membrane. A cell in complete equilibrium with its surrounding environment is dead.

Part B:
The Effect of an Enzyme on Diffusion

PROCEDURE

Collect about 10 ml of saliva in a test tube marked at the 10-ml level by a wax pencil.

Add this saliva to a piece of cellophane tubing, with one end tied.

Add 10 ml of soluble starch solution to the tube.

Place the cellophane tubing and its contents in a bottle (again as in B of the drawing). The bottle should not be too large—just large enough to hold the cellophane bag. Fill the

bottle with water at a temperature of about 45° C.

After about 15 minutes take two 5-ml samples of water from the bottom of the bottle with a clean pipette and put each into a test tube. Test the contents of one test tube for starch, the other for sugar.

For the starch test add a drop of iodine to the 5-ml sample in the test tube and observe the color change if starch is present.

For the sugar test add sufficient Benedict's solution to the 5-ml sample in the test tube to turn it blue. Put the test tube in the test-tube holder and heat it gently over the flame of the Bunsen burner. (CAUTION: *Do not point the mouth of a heating tube toward anyone*—the boiling liquid may boil from the tube if too vigorously heated.) A yellow or red precipitate forms if sugar is present.

Explain the results briefly. (11)

Part C:
Diffusion Pressure

Fill a piece of cellophane tubing (after tying one end tightly) with glucose solution. With a rubber band fasten the filled tube very tightly to a 1-ml pipette.

Fasten the pipette by means of a clamp to a ring stand, with the bottom of the filled cellophane tubing suspended in a container of water as shown in Figure 6-1-1C. Place the meter stick alongside the pipette to serve as a scale.

Record the position of the rising column of water measured by the meter stick at 2-minute intervals. Plot the data on a graph.

Explain the results briefly. (12) Remember that this model has demonstrated principles of diffusion which can be applied to situations in living cells.

ACTIVITIES OF

THE CELL MEMBRANE

Living material remains alive as long as it is able ·to maintain a proper chemical balance with its surroundings. If any part of the balance is upset the activity of the organism is disturbed, the organism shows signs of stress, and it may even die.

The membrane of the living cell has the vital role of regulating the passage of materials into and out of the cell. The membrane normally is selective and allows only certain substances to enter and leave the cell. The selective action of the membrane allows the cell to maintain, within certain limits, a chemical balance with the surrounding materials.

We will observe in this study, first the selective action of the yeast cell membranes to a certain dye; and second, the consequences of a chemical imbalance upon an elodea (*Anacharis*) cell.

■ The purpose of Part A of this exercise is to demonstrate the selective permeability of a living membrane. The purpose of Part B is to observe the consequences of a chemical imbalance between a living cell and its surroundings.

Part A:
Selective Action of Yeast Cell Membranes

PROCEDURE

Place 1 ml of yeast suspension in each of two test tubes.

Add 3 drops of Congo red solution to each test tube.

Heat the contents of one test tube over a flame until the boiling point is reached.

Study a drop of each of the two suspensions with high-power magnification.

Which yeast cells seem to be dead? (1) Do you notice any difference between the appearance of the dead and the living yeast cells? (2)

How do you account for this difference? (3)

Part B:
Consequences of a Chemical Imbalance Within a Cell

Elodea cells require a sodium chloride (table salt) concentration of approximately 0.9%

MATERIALS

Part A

5 ml suspension of yeast cells (freshly prepared) for each group of two to four students
Congo red solution
Slide and cover glass
Two test tubes
Test-tube racks
Compound microscope
Bunsen burner
Test-tube holder
Pipette

Part B

Sprig of elodea
5% Sodium chloride (NaCl) solution
Compound microscope
Slide and cover glass
Paper toweling or filter paper
Medicine dropper
Glass of water
Glass for NaCl solution

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within the cell. This does not appear to be a very high concentration of salt, but is about the concentration which must be maintained inside many kinds of living cells. You can upset this balance by placing a high concentration of salt solution around the outside of the cells.

PROCEDURE

Place a leaf from a growing tip of elodea in a drop of plain water on a clean slide. Add a cover glass and study it under low power and high power. Review its structure as studied in Exercise 3-5.

Place a small piece of paper toweling or filter paper at one edge of the cover glass to draw the water off the leaf. Add a drop of the salt solution on the opposite side of the cover glass. It will be drawn under the cover glass by the action of the filter paper. Observe the effect on the cells as the water moves over the leaf.

What do you see taking place within the cells? Describe. (4)

Will the cells return to a normal condition if the leaf is washed and again placed in plain water? Remove the leaf and place it in a glass of water and record results after a period of 5 minutes. (5)

Will the plant die if allowed to remain in an unbalanced salt condition for 10 to 15 minutes? Test this question by placing the leaf in a container of 5% NaCl solution. Remove after 15 minutes and observe. What are the results? (6) How can you tell if the cell is dead? (7)

What have you observed in these two experiments about cell membrane activity? (8)

Why do you think the membrane in one instance inhibits the passage of a substance and in another instance not? (9) A number of theories, several of which are discussed in your textbook, have been offered in answer to this question. One generally accepted theory is that the size of the molecule concerned is itself a determining factor. Can you draw conclusions regarding the size of the molecules of Congo red and water? (10)

SOME SPECIFIC

COMPOUNDS IN CELLS

You know that biologists can study the chemical composition of cells by grinding up tissues and performing analyses of this material in the laboratory. Other techniques can reveal whereabouts in the cell certain specific compounds are located. For example, a cell can be treated with iodine solution and then observed under the microscope to see the location of starch.

MATERIALS (Parts A and B)	
Potato	Slides
Apple or other fruit	Bunsen burner or
Iodine solution	alcohol lamp
Benedict's solution	Razor blade or
Compound micro-	sharp knife
scope	Centimeter ruler

■ The purpose of this exercise is to determine whether starch and sugar in plants are found only in specific cells.

PROCEDURE

In this exercise we shall determine the location of starch and sugar in the food-storage cells of potato and apple tissues. Since these cells cannot be peeled or stripped from the plant, the parts of the plant containing them have to be cut thin enough to allow light to pass through the cells. A microtome can cut slices that are one or two cells thick (10 μ or less), but if you try to slice a potato as thin as that by using a knife even as sharp as a razor blade, you will have difficulty because the slices will buckle and crease.

A way of getting around this difficulty is to cut from the potato or apple a piece as shown in Figure 6-3-1, and then to shave the piece into a wedge just as thin on the end as possible. The thin edge of the wedge should be only a few cells thick—you may need some practice to cut a thin wedge.

The wedge can then be placed on a microscope slide, and the thin edge may be observed under low power without a cover glass. If you wish to observe the cells under high power, you must cut off a very small piece of the thin
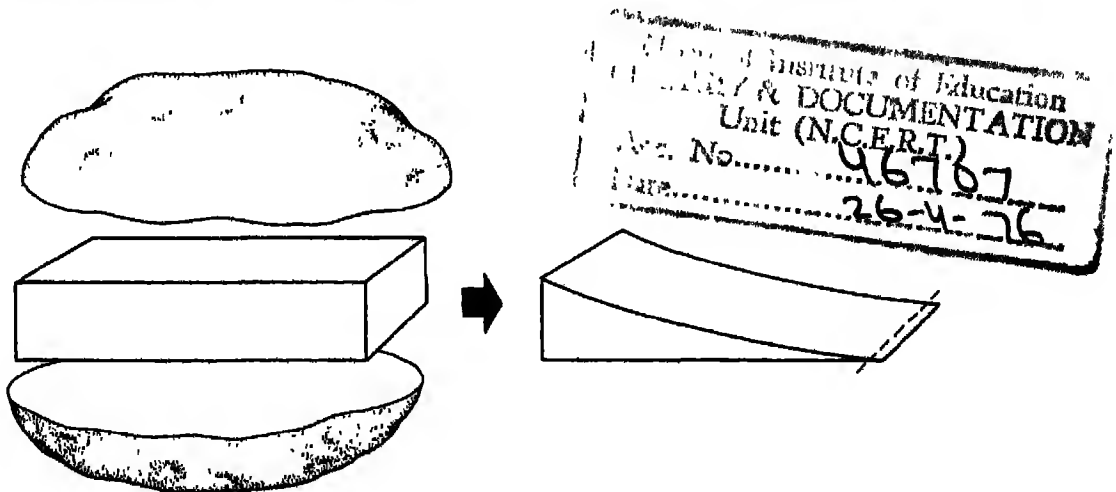
Figure 6-3-1 Freehand wedge section.

edge. Otherwise your mount will be thicker than the working distance of the high-power objective. It will not be necessary, however, to use high power this time.

Part A:
Starch in Potato Cells

Prepare a wedge of raw white potato by the method just described and observe the potato cells under low power.

Note the many round, colorless bodies within the cell and those spilled out of cells that were broken when the potato was cut. Draw a number of these bodies to bring out their differences in size and shape.

To determine whether these bodies contain starch, place a drop of dilute iodine solution on the edge of the potato wedge. Describe what changes occur in the potato wedge. (1) Why do not *all* the bodies under your microscope react the same way to the iodine? (2) Does this test show that the potato contains only starch? (3)

Part B:
Sugar in Apple Cells

Prepare a wedge of apple or some other fruit by the method described above and mount it on a slide.

Add a drop or two of Benedict's solution and examine the cells under the microscope. Make a sketch of a few cells, so you can later compare them with cells *heated* in Benedict's solution.

Remove the slide from the microscope and heat it gently over the flame of a Bunsen burner or alcohol burner. Be careful not to get the slide so hot that it cracks.

Allow the slide to cool and re-examine the cells under the microscope.

What changes have taken place in the cells? (4) Is sugar in special cells in the apple? (5) Compare the location of starch in the potato with that of sugar in the apple. (6)

Now, in the manner described above, test the potato for sugar. Is the test positive? (7) Test the apple for starch. Is there starch in the apple? (8)

ENZYMES IN LIVING TISSUE

By this time you have discovered that enzymes are very important in living organisms, where they control nearly all chemical reactions. They are **biocatalysts** that are made up of proteins and they accelerate the rate of reactions. Many of them are easily isolated from organisms and are used to catalyze chemical reactions outside the body of the organism, while many others are more difficult to obtain. The following experiments are to demonstrate the activity of enzymes in living tissue.

MATERIALS

Part A
Test tube
Test-tube holder
Beaker
Funnel
Forceps
30% hydrogen peroxide (H₂O₂) solution
Bunsen burner
A variety of animal and plant tissues: potato, frog blood, fly, worm, etc.
Mortar and pestle
Filter paper
0.1 M Sodium hydroxide (NaOH)
0.1 M Hydrochloric acid (HCl)
Pebble, marble, or other inert substance
Graduated cylinder

Part B
Three Petri dishes containing starch agar
One Petri dish containing plain agar
Dish of soaked corn grains
Dish of soaked grains killed in formaldehyde, alcohol, acetic acid (FAA)
Iodine solution
Sharp razor blade

■ The purpose of this exercise is to show the presence of an enzyme, known as catalase, in different kinds of cells and also to demonstrate the presence of a starch-digesting enzyme in living plant cells.

Part A:

Enzyme Activity in Various Tissues

In this experiment you will have an opportunity to see that many different kinds of cells contain a common enzyme called **catalase**. You will then see catalase facilitate a simple chemical reaction in a test tube, as it does in living cells.

Hydrogen peroxide is a highly active chemical useful for bleaching or for cleansing minor wounds. It is formed in living systems. If it were not immediately removed or broken down it would destroy the living system. Catalase immediately reacts with hydrogen peroxide by breaking it down into harmless water and oxygen.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

PROCEDURE

Place a piece of clean filter paper on your desk next to the beaker.

Put 5 ml of hydrogen peroxide solution into a test tube.

Using your forceps, select several small pieces of animal or plant tissue from the demonstration table, and place them on the filter paper until you are ready to use them.

With your forceps drop a piece of tissue into the test tube of hydrogen peroxide. What reaction occurs? (1)

Remove this tissue and add a different tissue to the tube. Is the reaction the same as the first? (2) Remove this tissue and repeat with several others.

Make a chart listing on the left-hand side the names of the tissues used and indicate for each tissue the reaction observed in hydrogen peroxide solution. Are all the reactions equal in amount? (3)

Treat pieces of the same tissue used above by boiling them; or by grinding them; or by mixing them with water, and then filtering and testing the filtrate; or by soaking them in 0.1 M

HCl or 0.1 M NaOH (CAUTION: *Use care in handling HCl and NaOH! Never let them get on skin or clothing*). Write the type of test used on the tissue along the top of your chart and record the reactions for each tissue in the body of the chart. Are the reactions the same with the treated tissues as with the untreated tissues? (4)

Put a small pebble, marble, or other inert substance into the hydrogen peroxide solution. Is there any reaction? (5)

If you were told that the bubbling of the materials you are testing in hydrogen peroxide indicates the presence of catalase, what would you conclude about catalase from the above experiment? (6)

What purpose does the test of inert materials serve? (7) In a scientific experiment, what do your recorded observations constitute? (8) When you attempt to account for your observations, what may your statement regarding them constitute? (9)

Part B:
Enzyme Activity in Corn Grains (Demonstration)

You may have noticed that certain vegetables and fruits vary in taste. Some carrots are rather tasteless, while others are very sweet; with increasing age a banana changes from a rather starchy-flavored to a sweet-flavored fruit. In each case it is obvious that in the sweet-tasting fruits and vegetables additional sugar is formed from insoluble carbohydrates. The following demonstration will show that enzymes capable of digesting starch to sugar are present in living tissues.

PROCEDURE

Take a soaked (germinating) corn grain, cut it lengthwise with the razor blade, and test the cut surfaces with iodine solution. What food is present in abundance? (10) What other foods known to be present in corn are not demonstrated by the test? (11)

Your teacher will take one of the Petri dishes containing starch agar and one with plain agar and flood them with iodine solution. What difference do you observe? (12)

On another Petri dish of starch agar there are two or three corn grains which have started to germinate. Each has been cut lengthwise and the cut surface placed on the agar. They have been on the agar for about 2 days. On a third Petri dish containing starch agar are cut corn grains which had started germinating but which were killed before they were put on the agar. The cut corn grains will be removed from the surface of the starch agar by your teacher, who will flood the surface of each dish with iodine solution for 2 or 3 minutes and then pour off the excess.

How do you account for the difference in the areas in the agar on which germinating corn grains were located? (13) What kind of food would you expect to find in the clear areas? (14) What do you observe in the Petri dish which contained germinated but killed corn grains? (15) Would dry corn grains work as well as moist, germinating ones? (16)

ENZYME ACTION

ON A PROTEIN

Enzymes are proteins which catalyze (speed up) chemical changes. Hundreds of different kinds of enzymes are present in the cells of plants and animals. Each kind of enzyme is specific; that is, it acts on a particular substance to bring about a particular change.

In the laboratory we can demonstrate the actions of certain enzymes on different materials. In this exercise you are to determine the action of an enzyme on albumin, a protein found in egg white. We shall use the enzyme, bromelin, which is found in quantity in fresh pineapple juice. If an enzymatic action takes place, the albumin will be hydrolyzed to amino acids. You will test for amino acids by means of a technique known as **paper chromatography**.

Chromatography means literally "color writing." By this technique the components of a mixture are separated in a relatively easy and quick manner. The technique was first used for the separation of pigments on paper; hence the name paper chromatography. This procedure was later applied to separate colorless substances to which sprays could be applied to produce a color, thus developing what are called **chromatograms**. (Column chromatography is a technique by which substances are separated by passing them through a suitable absorbent material within a glass tube.) Paper chromatography is still the most popular because it is inexpensive and easy to perform.

In chromatography, certain substances in mixtures are separated by an adsorption process and by capillary action. (**Adsorption** is the attraction of unlike molecules for each other. **Capillary action** is the movement of liquids in narrow spaces, such as tiny tubes.) In our study we will use a simple paper-strip chromatographic technique. A drop of solution containing a mixture of substances to be tested is placed on a strip of filter paper near one end. This end of the paper strip is placed in a suitable solvent within a closed container. The solvent ascends the strip by capillary action, like ink spreading in a piece of blotting paper. When the solvent reaches the spot of the mixture, the mixed substances are carried along the paper. Each substance of the mixture will

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MATERIALS (Parts A and B)

Test I mixture— two pieces hardboiled egg white in 2 mm cubes, 10 ml frozen or fresh pineapple juice, 3 drops of toluene (CAUTION: *poison*) as a preservative

Test II mixture — same as Test I except that the pineapple juice is boiled for 10 minutes, or canned pineapple juice is used [The two mixtures have been prepared and allowed to set for approximately 48 hours before the test is performed.]

Two large test tubes for each group of students

Four corks to fit test tubes

Forceps

No. 1 Whatman chromatography paper or filter paper

Cellophane tape

Ninhydrin reagent

5-ml or 10-ml pipettes

Solvent of butanol, glacial acetic acid, distilled water (4 : 1 : 1)

Toluene

Wax pencil

Test-tube rack

Gelatin

Scissors

Bowl for ninhydrin reagent

Bunsen burner

have a characteristic, usually different, affinity for the solvent and the paper. The substances will adsorb (or cling) to the cellulose fibers of the paper with different degrees of force, and so different substances will move along with the solvent at different speeds. After some length of time the substances of the mixture will become separated from each other along the path of the solvent. In this exercise we will not attempt to analyze or separate any particular amino acids which may be produced.

■ Our purpose is simply to determine whether the protein (albumin) has been broken down into amino acids by the action of the enzyme (bromelin) from pineapple juice and to familiarize ourselves with the technique of paper chromatography.

Part A:
Paper Chromatography

PROCEDURE

1. Prepare the chromatographic strip by cutting a strip of chromatography paper or filter paper to fit into each of two test tubes (see Figure 6-5-1). At all times handle the strip of paper with forceps, since fingerprints leave amino acids on the paper. Tape or tack one end of the paper strip to the bottom of the cork or cut a slit in the cork and insert the top of the paper into it. At the opposite end of the strip trim the edges to a point, as shown in the drawing. Place the cork and strip in the test tube, making certain that the point of the strip is slightly above the bottom of the tube, and that the edges of the strip do not touch the tube at any point. Trim the strip if necessary. With a wax pencil make a mark on the outside of the test tube at a point approximately 1 cm above the tip of the strip.

2. Remove the corks and strips from the test tubes. Pour the solvent (CAUTION: *The solvent is a poison*) into the test tubes to the marked line. Stopper the test tubes tightly with the second corks, place them upright in a test-tube rack, and leave them until the next preparation is made. This allows the air within the test tube to become saturated with the solvent.

3. Gently shake the containers of the test mixtures. Use a clean pipette to place a small drop of the Test I mixture on one paper strip approximately 2 cm from the pointed end, as shown in the drawing. Write with a pencil at the top of the strip, TEST I. In the same manner transfer a drop of Test II mixture to the second strip and label this strip TEST II. Allow the drops to dry for 3 to 5 minutes and then apply a second drop of the same test mixture over the first drop and allow it to dry again.

4. When the drops are completely dry, uncork the test tubes and place in each tube the cork and strip prepared in steps 1 and 3 above. Take care that the strips do not touch the sides of the tubes and that the lower end of the paper strip is barely immersed in the solvent. Set the tubes in the rack for approximately 1 hour and observe the rise of the solvent.

5. When the solvent has ascended to within 20 mm of the top of the strip, remove the cork and the strip from the tube. Mark on the strip with a lead pencil the highest point the solvent has reached. Remove the paper strip from the cork and hang it by its tape in a convenient place to dry until the following day.

6. The following day, develop the strips (which we now call *chromatograms*) with the ninhydrin reagent. (CAUTION: *Ninhydrin is a poison. Take care that fumes are not inhaled.* If a fume hood is available, it is best to use it when working with ninhydrin to minimize odor and risk.) This is done by placing the reagent in a flat bowl and immersing the strip, *held by forceps*, in it briefly. Dry the strips by holding them several inches above a low flame. Take care that the strip does not burn. If amino acids are present a purple color will appear in one or more spots along the strip. If the paper was handled carelessly color spots from fingerprints may appear along the edges of the strips.
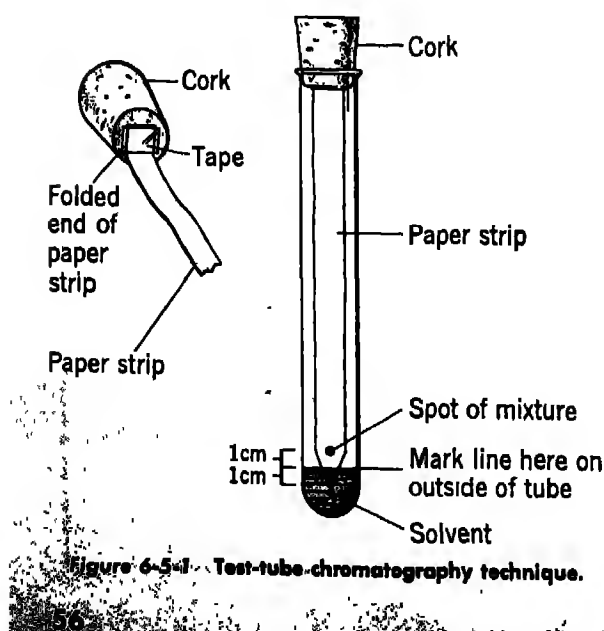
Figure 6-5-1. Test-tube chromatography technique.

Cork

Cork

Tape

Folded end of paper strip

Paper strip

Paper strip

Paper strip

Spot of mixture

1cm
1cm

Mark line here on outside of tube

Solvent

Does the Test I chromatogram show spots of color indicating the presence of amino acids in the test mixture? (1) Does the Test II chromatogram indicate the presence of amino acids in the second mixture? (2) What conclusions can you draw from these tests? (3)

Part B:
Action of an Enzyme on Gelatin

It is common knowledge among housewives that gelatin will not gel or harden when mixed with fresh pineapple. On the basis of your results in Part A, form a hypothesis as to why this should be true. (4) What kind of food substance do you conclude gelatin to be? (5) Can you guess why it is all right to use canned pineapple juice or cooked pineapple in gelatin? (6)

You can demonstrate this effect very simply in the laboratory. Dissolve 4 g of gelatin in 20 ml of hot water. Divide into two portions. To one portion add 2 ml of undiluted frozen pineapple juice. To the other portion add 2 ml of canned or boiled pineapple juice. Allow to set for 10 minutes. Describe the differences between the two. (7) How do you account for the difference? (8)

FACTORS INFLUENCING

ENZYME ACTION

We now turn to a study of enzyme activity outside the living organism. We shall use an enzyme (amylase) found in ordinary saliva. By varying the conditions of the experiments we can learn how the enzyme works. In each of the following studies we introduce variables, one at a time, that may influence the action of salivary enzymes.

■ The purpose of this exercise is to determine the influence of temperature, time, dilution, and pH upon the action of an enzyme.

MATERIALS (Parts A, B, C, and D)

Iodine solution	Two test-tube
Benedict's solution	holders
0.1 M Sodium hy-	Test-tube rack
droxide (NaOH)	Funnel
0.1 M Hydrochloric	Filter paper
acid (HCl)	Thermometer
Soda crackers	500-ml beaker
(sugar-free)	250-ml beaker
Bunsen burner	100-ml beaker
Ice	Slides or watch
Paraffin	glass
Starch solution	1-ml pipette
Tripod stand and	Wax pencil
screen	10-ml graduated
Four to sixteen	cylinder
test tubes	

Part A:
The Action of Salivary
Enzymes at Various Temperatures

In this exercise you will be asked to record temperatures using the Celsius scale, rather than the Fahrenheit scale. Almost universally in science, temperature is measured in degrees Celsius (C). In Appendix II there is a discussion of the Celsius—Fahrenheit temperature relationship as well as a drawing of parallel temperature scales so that temperatures on one scale can be converted directly to the other scale.

Experiment 1: Enzyme Action at Room Temperature

1. Crush a fragment of a cracker and mix it with water in a test tube.
2. Test a portion of the mixture for starch by adding a drop of iodine solution. A blue color indicates that starch is present.
3. Test another portion of the mixture for sugar by adding a few drops of Benedict's solution in a test tube and heating it.
4. Record your results in the table, Starch and Glucose Test Results, or in a similar table that you make in your notebook. Use a "+" if the material tested for is present and a "−" if the material tested for is absent.
5. Chew paraffin and collect some saliva in a test tube. Test it for sugar and starch, as in steps 2 and 3 above. Record your results in the table.
6. Two students are to chew bits of cracker with plenty of saliva and deposit the chewed cracker in a funnel lined with folded filter paper. Add a little warm tap water and collect the filtrate in a test tube.
7. Test the filtrate for starch and sugar, as in steps 2 and 3 above, and record in the table.

Does the enzyme amylase change starch into something else? (1) What is the material produced by the action of amylase on starch? (2) Does the starch solution pass through the filter paper? (3)

Suggest a reason for the result noted in your answer to Question 3. (4) Remember that these same principles demonstrated in test tubes and beakers operate also in living organisms.

Experiment 2: The Effect of Temperature on Enzyme Activity

1. Chew some paraffin to stimulate the flow of saliva and collect the saliva in a test tube. Some students should boil the saliva, cool, and then add a few bits of cracker. Other students should heat the saliva to 60° C and still others heat it to 37° C. Set the tubes aside for about 10 minutes.

2. At the end of 10 minutes test for glucose as in step 3 of Experiment 1. Record the result. Are the tests positive? (5) How do you account for the results? (6)

3. Chew paraffin and collect the saliva in a test tube placed in a beaker containing pieces of ice.

4. After 5 minutes add fragments of cracker to the saliva.

5. After 10 minutes test the iced saliva and cracker mixture for glucose as in step 3 of Experiment 1. Record your observations in the table.

Make a statement regarding the effect of temperature on enzyme action as observed here. (7)

Part B:
The Speed of Salivary Enzyme Action

1. Pour about 3 cm of warm water into a 250-ml beaker and place this on the screen on top of the tripod stand over your heat source. Carefully place a thermometer in the beaker and adjust the heat to keep the water at body temperature (37° C).

2. Pour about 10 ml of starch solution into a 100-ml beaker and place this in the larger beaker of warm water. Continue to apply low heat to maintain the temperature.

3. Chew some paraffin to stimulate the flow of saliva and collect filtered saliva in a test tube or beaker. Be ready to test for starch and sugar.

4. Now add the saliva to the starch solution and mix thoroughly.

5. Immediately after adding the saliva and each 2 minutes thereafter, a few drops of the solution should be tested for starch by one student and a 2-ml portion should be tested for glucose by another student.

a. To test for starch, transfer a drop to a slide, add a drop of iodine solution, and stir if necessary. Record the color resulting from the test and the significance of the color. Clean the slide for the next test. As the test is repeated every 2 minutes, watch for the point where the test no longer reveals starch.

b. Test for glucose every 2 minutes to detect the progress of digestion. Put about 2 ml of the starch-saliva solution into a small test tube with a few drops of Benedict's solution. These must be washed by swirling to the bottom of the test tube and heated. Read the results quickly and record the resulting color and its meaning.

6. From your records determine the time required to reach the point where starch has been digested and where sugar is present. Do enzymes react slowly or rapidly? (8)
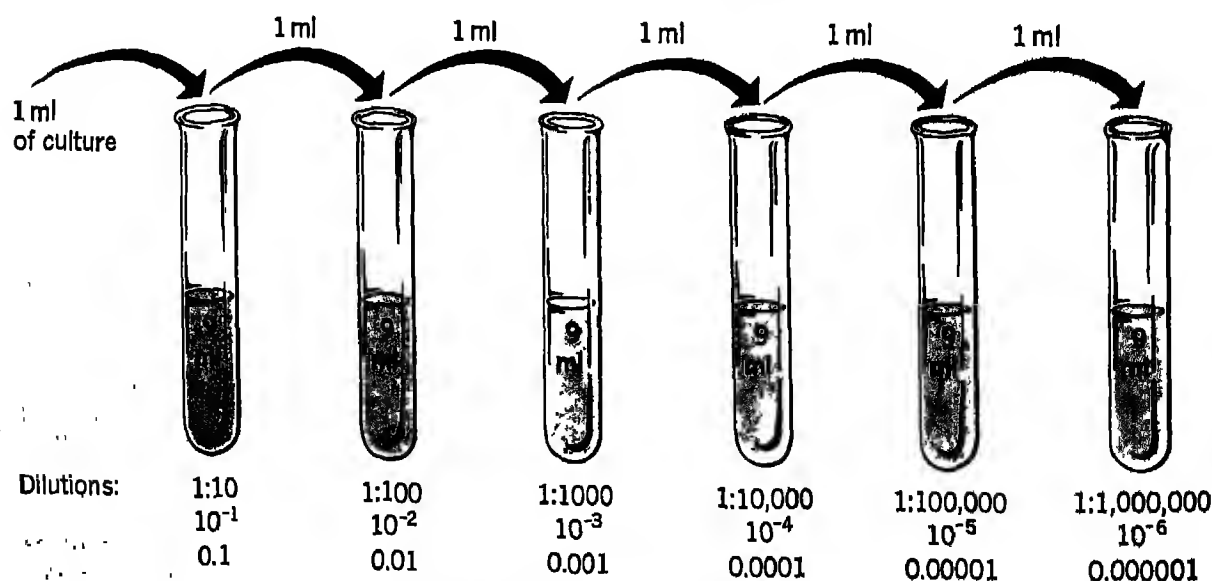
Figure 6-6-1 Enzyme dilution method.

| Dilutions: | 1:10 10^{-1} 0.1 | 1:100 10^{-2} 0.01 | 1:1000 10^{-3} 0.001 | 1:10,000 10^{-4} 0.0001 | 1:100,000 10^{-5} 0.00001 | 1:1,000,000 10^{-6} 0.000001 |

Part C:
The Effect of Dilution
on Enzyme Activity

1. Fill a 500-ml beaker about ⅛ full of water, and warm it gently to body temperature (about 37° C).

2. Collect about 1 ml of saliva in a test tube.

3. Number six test tubes 1 through 6 with a wax pencil. Pour 9 ml of water into each of the test tubes. Add the 1 ml of saliva to the first tube. Mix thoroughly by shaking. This, of course, gives a 1:10 dilution.

4. Take 1 ml of solution with a pipette from Tube 1 and transfer it to Tube 2. Mix, then clean the pipette and transfer 1 ml from Tube 2 to Tube 3. Mix, clean the pipette, and continue this process until Tube 6 is reached, as shown in Figure 6-6-1.

5. Add 1 ml of starch solution to each test tube, mix thoroughly, and place the tubes in the warm water bath that you prepared in step 1.

6. After 10 minutes one member of the team should withdraw a small sample from each tube and test it with iodine solution for the presence of starch, as you did in step 5a of Part B.

7. The second member of the team should test a 1-ml sample from each tube for sugar, as in step 5b of Part B.

8. Repeat the tests for starch and sugar (steps 5a and 5b in Part B) 20 minutes after the beginning of the experiment.

9. Record the results in tabular form. Write a paragraph explaining the effect of dilution on enzyme action. How much may this enzyme be diluted and still be effective? (9)

Part D:
The Action of an Acid
and a Base on Enzyme Action

1. Chew some paraffin to stimulate the flow of saliva and collect this saliva in a test tube or beaker. You will need about 12 ml of saliva.

2. Prepare a warm water bath as in step 1 of Part C.

3. One student of the team now prepares a series of dilutions of HCl starting with the 0.1 M solution provided. Number six test tubes 1 through 6 with a wax pencil. Place these numbered tubes in order in a test-tube rack and place 10 ml of the 0.1 M solution in the first tube. Place 9 ml of water in each of the other tubes. Transfer 1 ml from Tube 1 to Tube 2 and continue through the six tubes, as in step 4 of Part C.

4. A second member of the team should dilute the NaOH solution to secure dilutions of this base similar to those obtained for the acid in step 3 above.

5. Prepare a seventh tube with 10 ml of water to serve as a control. Place all seven tubes in the warm water bath as they are prepared.

6. Add 2 ml of the starch solution to each tube. Now add 1 ml of saliva to each tube and mix thoroughly. After 10 minutes test samples from each tube for the presence of starch and sugar.

7. Record your results in tabular form. Write a paragraph explaining the effect of various concentrations of an acid and a base on enzyme action. (10)

Describe what factors influence enzyme action and how they influence it. (11)

STARCH AND GLUCOSE TEST RESULTS

Materials Tested	Starch Test	Sugar Test
Cracker		
Saliva		
Cracker and saliva filtrate		
Room temp. = _____° C		
Boiled saliva with cracker		
Saliva heated to 37° C		
Saliva heated to 60° C		
Saliva and ice with cracker		

MITOSIS IN PLANT
AND ANIMAL CELLS

"Like tends to beget like." This phrase has a meaning so self-evident, we hardly pause to give it a second thought. We know that when oak trees reproduce they give rise to oak trees; when rabbits reproduce there are more rabbits. Somehow the reproductive cells of an oak or a rabbit have received hereditary materials that give them specific characteristics of oaks or rabbits and not of some other organism.

How have these hereditary potentialities been passed on from one cell to the next in such a precise way that *all of them*, both in *quantity* and *quality*, can be transmitted to the reproductive cells?

This precise method of transmittal of hereditary potentialities from one nucleus to the next in body cells of an organism is accomplished by mitotic division. For the hereditary potentialities are the results of the action of genes located in the chromosomes within the nucleus of each cell.

Mitosis is essentially the same in principle in both plant and animal cells. Only the details are different. Remember not to overemphasize the differences in detail.

The process of mitosis is not easy to see in living cells because the nucleus and all the structures within it are colorless and nearly transparent in the living condition. We learned in an earlier exercise about a special kind of microscope—the phase-contrast microscope—that makes it possible to see cell structure without killing cells and that makes it possible to observe mitosis in living cells.

It is customary to look at sectioned and stained preparations of plant or animal cells to see nuclear and cell divisions. The methods involved were discussed in Exercise 3-9. These preparations have one obvious disadvantage, that which results from the sectioning of the dividing nuclei. As a result, in any one section we see only some parts of a dividing cell or nucleus. By using the *smear* or

squash technique we can see complete cells and nuclei in division. In this exercise you will be able to see both smears and sections of dividing cells.

■ The purpose of this exercise is to show mitosis in plant and animal cells and to provide an understanding of the dynamic events of chromosome division that produce two identical cells from one.

MATERIALS

Part A
Compound microscope with all lenses and the mirror as clean as you can get them
Tradescantia flower buds
Aceto-carmine stain
Rusty, single-edged razor blade
Forceps and dissecting needle
Clean slide and cover glasses
Millimeter ruler
Paper toweling

Part B
Fresh onion bulbs 5 to 7 cm in diameter
Bottles or dishes for growing roots from onion bulbs
Toothpicks
Aceto-carmine stain
1 M Hydrochloric acid (HCl)
Rusty, single-edged razor blade
Dissecting needle and forceps
Clean slide and cover glass
Paper toweling
Watch glass

Part C
Prepared slide of onion root tip

Part D
Prepared slides of *Ascaris* and/or whitefish eggs

Part A:
Smears of Developing Pollen

PROCEDURE

The class will be divided into groups. Each student in a group is to select a flower bud of *Tradescantia* (fresh or preserved) ranging in size from 2 to 8 mm in length. Measure from the tip to the point of attachment of the flower stalk. One student from each group should select a bud 2 mm long, the next student a 3-mm bud and so on up to and including the 8-mm bud. Out of this selection at least one member of the group will obtain a bud whose pollen shows stages in mitosis.

Now remove the pollen sacs from the buds. Put the flower bud on a clean slide lying on a piece of paper toweling. Use the forceps and dissecting needle to open the bud and tease out the pollen sacs. In fresh flower buds these are light to bright yellow and occur in six pairs, each about the size of the double period at the end of this sentence (..).

Temporarily put five of the six pairs of pollen sacs in a drop of water (do not let them dry out) on the end of a slide. Put one set of pollen sacs on the middle of the slide and add a drop or two of aceto-carmine stain. Squash open the pollen sacs in the stain with the dissecting needle, cut them up with the old razor blade, and remove and discard the larger fragments of the pollen sacs from the stain. You will not need them again. To complete this squash or smear, put the cover glass over the stain containing the smaller fragments of the pollen sacs. Tear a piece of paper toweling slightly larger than the cover glass and put this on top of the cover glass. Using the eraser end of a pencil, push down firmly on the towel-covered cover glass several times in different places so that the excess stain squeezes out and is absorbed by the towel.

Look at the stained cells under the low power of the microscope. You will see oval cells (pollen grains) stained pink with parts of the nucleus appearing deeper red. When you have identified the pollen grains switch over to high power and look for cells in which the nuclei are dividing. They can be easily identified because the stained material of the nucleus is condensed into threads or distinct linear bodies called **chromosomes**—the bearers of hereditary material.

Do not be disappointed if your specimens do not show mitosis. The bud you used may have been too young or too old. But one member of your group should have prepared pollen sacs which are of the right age. This student will give each of the other members of the group one of the five pollen sacs that were placed on the end of the slide and saved to be made into smears. These will contain stages in mitosis. Find four stages of mitosis:

a. A stage in which the nuclear material forms long, slender, stained threads. The separate chromosomes cannot be identified because they form a loose ball of tangled and twisted threads.

b. A later stage in which the individual chromosomes are much shorter and thicker and can be easily identified. How many are there in dividing pollen grains of *Tradescantia*? (1) At this stage each of the chromosomes is clearly composed of two parallel strands (daughter chromosomes) held together at one point by a nonstaining structure called a centromere. How can you explain the presence of two parallel and duplicate strands in each chromosome? Is this a result of splitting of the chromosomes or a replication of the chromosome? (2)

c. A stage in which daughter chromosomes have separated and are arranged in two separate groups. How many daughter chromosomes are there in each pollen cell at this stage? (3) Is the division of the nucleus, *as you see it*, a qualitative or a quantitative replication? (4)

d. A stage in which the two daughter nuclei are present in the pollen cell, but strands representing daughter chromosomes are still visible. Draw four stages of mitosis in *Tradescantia* pollen. Draw a single chromosome as seen in stage b. Compare it in shape and size with the other chromosomes. (5)

Part B:
Smears of Onion Root Tips

You can make preparations of onion root tips. The technique is similar to the one used above for *Tradescantia*. Your teacher will be able to tell you how to take the onion root tips and how to make the slides.

Part C:
Prepared Sections of
Onion Root Tips

Aceto-carmine does not stain certain important structures in the mechanism for the separation of daughter chromosomes and the estab-

lishment of a new cell wall in cell division.

Therefore it is customary to look at sectioned and stained preparations of plant or animal cells to see nuclear and cell divisions. In this part of the exercise we shall look at prepared sections of dividing cells in the onion root tip, to supplement our observations of smears.

PROCEDURE

Scan the entire length of the slide to observe that cells far from the tip and cells right at the tip are not dividing. Locate the area of cell division between these two regions. Switch to high power and look for the following:

a. A stage showing early appearance of the chromosomes within the nucleus. Find spherical bodies (one or two) which stain at this early stage. These are the nucleoli (singular—nucleolus.

b. A stage in which the chromosomes are easily visible and are grouped in the middle of the cell. Are nucleoli still present? (6) Is there any sign of a nuclear membrane at this stage? (7) Find the spindle, which is composed of threads radiating from the poles (opposite ends) of the cell to the chromosomes at the equator (middle) of the cell. Spindle fibers from each pole are attached to the centromeres of the chromosomes at this stage. Can you count all of the 16 chromosomes in this section? (8) Do they appear to be double? (9)

c. A stage in which the daughter chromosomes have separated and have moved toward the poles of the spindle. What relationship do you think the spindle might have to the separation and movement of chromosomes? (10) Draw one stage in mitosis showing a dividing nucleus with the spindle and chromosomes.

d. In the division of many kinds of plant cells a new cell wall is formed between daughter cells. Look for the beginning of this wall. Where in the mother cell does it appear? (11) In what way is it related to the spindle? (12)

Part D:
Animal Cells in Mitosis

Because animals also begin life as a single cell (the fertilized egg) and because great numbers of such eggs can be obtained from sea animals such as sea stars (starfish), sea urchins, and fishes, it is easy to observe stages of mitosis in the cells making up the young embryo of one of these animals. The developing eggs of the parasitic worm *Ascaris* are also often used. The eggs can be killed and stained by procedures very similar to those used for the plant tissues. Examine carefully a prepared slide of developing *Ascaris* or whitefish embryos.

Find a cell in which the chromosomes are long and threadlike. Can you count the individual chromosomes in *Ascaris* eggs? (13)

Find an *Ascaris* cell in which the replicating chromosomes are attached to the equator of the spindle. Look at the poles of the spindle and compare them with those of the plant cells you have previously studied. How do they differ from those of the plant? (14)

Find a cell in which the daughter chromosomes are separating and the cell is pinching in two. Compare this method of the separation of the daughter cells with that customary in plant cells. (15) What structures do you see in the dividing animal cell that were also present in the dividing plant cell? (16) What structures do you see that were *not* in the dividing plant cell? (17)

HELPFUL BACTERIA

We commonly hear of harmful bacteria. However, bacteria may not only be helpful, but they can actually be essential in the community of living organisms.

Many organisms live together in ways in which one or both partners are helped. The general term for living together is symbiosis. In this exercise we will investigate a type of symbiosis in which both partners benefit. This type of symbiosis is called mutualism.

In leguminous plants such as clover, the plant root is invaded by bacteria and little lumps or nodules form on the plant roots. These bacteria serve to fix nitrogen in a form usable by the plant. Of what benefit to the legume is the nitrogen-fixing bacterium? (1) What benefit does the microorganism derive from this association? (2)

Review Chapter 8 of your textbook for the role of nitrogen-fixing bacteria in the nitrogen cycle.

■ The purpose of this exercise is to understand the role of bacteria and the value of nitrogen fixation in the nitrogen cycle.

MATERIALS

Part A
Leguminous plants
India ink or methylene blue
Two dissecting needles
Compound microscope
Slide and cover glass

Part B
Three 5-inch flowerpots with saucers
Washed sand
Commercial *Rhizobium* inoculum supplied
  with specific legume seeds
Nodules from roots of legume
Zonite or other fungicide (1:10 dilution)
Distilled water
Test tube
Stirring rod
Nitrogen-free nutrient

Part A:
The Nitrogen-fixing Bacteria

PROCEDURE

Wash the roots of a legume carefully with water, being careful not to remove the root nodules.

Place a nodule in a drop of water on a slide and crush it. Remove the larger parts of the nodule and leave the remaining milky material on the slide. Cover with a cover glass and observe under the low and high powers of a microscope.

Add a drop of India ink or methylene blue to stain the material. Observe again and record your observations. The microorganisms seen here are *Rhizobium* bacteria. Have they all the same shape? (3) Make outline drawings of the bacteria present. Are these bacteria producing a disease in the plant? (4)

Beijerinck, who discovered these nitrogen-fixing microorganisms in the roots of plants, hesitated to call them bacteria but called them "bacteroids" (bacterialike structures). Assuming that he saw what you see, what reason might he have had for this hesitation? (5)

Part B:
The Role of
the Nitrogen-fixing Bacteria

PROCEDURE

Sterilize enough sand to fill three flowerpots, by autoclaving it or by pouring boiling water over it several times.

Cool the sand and fill the three pots. Number the pots of sand from 1 through 3.

Wash the legume seeds with a 1:10 dilution of fungicide for 5 minutes. Rinse the seeds two or three times with distilled water and allow them to dry.

Crush some root nodules and place them in a test tube containing 3 ml of distilled water.

Stir with a stirring rod until the water looks milky. This is your own *Rhizobium* inoculum.

In Pot 1 place five or six legume seeds.

In Pot 2 place five or six seeds that have been inoculated with the commercial inoculum. (To inoculate the seeds, sprinkle some of the inoculum on them before planting or pour some of the inoculum on the seeds after planting.)

In Pot 3 place a few seeds that have been inoculated with your prepared inoculum.

Water all of the seeds with the nitrogen-free nutrient; keep the sand moist at all times with the nutrient.

Place all of the pots in similar locations until the seeds have germinated, then place them in a well-lighted place.

Record the growth and appearance of the legumes in each of the three pots.

Which seeds germinate first? (6) Is there any difference in the growth patterns of the inoculated and the uninoculated legumes? (7) Which inoculum would you prefer if you were a farmer? (8) What is the reason for inoculating the seeds before planting? (9) Why do you suppose the seeds were washed with a fungicide? (10) How are root-nodule bacteria important to man? (11)

DIVERSITY

Microorganisms

Microorganisms are all around us even though we are not conscious of their presence. They are spread far and wide from those places where they grow (habitats) to all parts of our environment. Often they go along for a free ride with anything that moves. So the questions of where microorganisms are found and how they are spread are appropriate ones to ask and the answers will be found in the following exercises.

Microorganisms are literally at the mercy of their environment and may be affected favorably or unfavorably by very slight changes in their environment. An organism can exist only within definite narrow limits set by the nature of its surroundings.

Among the more important environmental conditions affecting microbial growth are temperature, moisture, oxygen, food supply, hydrogen-ion concentration, presence of certain chemicals, and the presence of other organisms. Our laboratory study will reveal the influence of some of these factors on microorganisms.

We will have an opportunity to learn techiques of handling and culturing organisms so small that they can be seen only with high-powered microscopes. We will also learn that microorganisms are not all harmful disease producers but that many are essential for our life on earth. Some do need to be controlled, however, and the laboratory work will acquaint us with ways in which the numbers of microorganisms can be reduced and the microbial populations controlled.

These exercises give us an opportunity to work with the very small and to understand microorganisms as an important part of our living world.

MICROBIOLOGICAL

TECHNIQUES

The next series of laboratory exercises will deal with those tiny creatures we group under the title "microorganisms." A microorganism is not a specific kind of animal or plant but simply one which is too small to be seen effectively with the unaided eye. Included here are such organisms as bacteria, yeasts, molds, protozoa, and some algae.

Most of the methods used to study microorganisms in general were first developed to study a particular group, the free-living bacteria, which are the smallest microorganisms.

Because you will be dealing with organisms that you cannot see, the techniques of handling and growing microorganisms must be mastered before any constructive laboratory work can be undertaken. Therefore, it is essential that you learn the methods by which microorganisms are handled and grown. Although the methods may seem complicated at first, actually they are simple to learn and essential for a study of microorganisms. The techniques can be mastered with a little practice.

■ The purpose of this exercise is to familiarize you with the techniques of handling microorganisms so that you will understand the problems of culturing and transferring them and so that you will be able to perform the work of the succeeding exercises.

PROCEDURES

Aseptic Techniques

In dealing with microorganisms, the glassware, needles, loops, and all materials used in handling and growing these organisms must be scrupulously clean. This does not mean simply washed in soap and water, which will free these objects of dirt visible to the naked eye. It means they must be sterilized to remove or kill all microorganisms that may be present. The technique of using materials which have no living microorganisms on them is called an aseptic technique.

Microorganisms can be removed or killed by the use of heat as in an autoclave, by flaming, or by chemical means such as the use of acids, alkalies, salts, and various other compounds such as alcohol and phenol. The chemicals used in sterilization of glassware and equipment are called disinfectants. Those used on living tissues, such as human skin, are called antiseptics and are not as powerful as the disinfectants. Antiseptics and disinfectants will either kill microorganisms or inhibit their growth. Would an antiseptic be more likely to kill bacteria or to inhibit their growth? (1) Why? (2)

When you work with microorganisms all equipment and culture media must be sterilized. However, when we transfer microorganisms from one piece of equipment to another

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MATERIALS

Inoculating loop
Inoculating needle
Pipette
Bunsen burner or alcohol lamp
Petri dish
Test tube
Autoclave
Incubator

Culture media (sterile agar, sterile slants in test tubes, sterile agar in Petri dish, sterile broth in flask)
Cotton for plugs
Chemical disinfectant (chlorine solution; benzalkonium chloride solution; Lysol or some other phenol preparation)

they are at least briefly exposed to an environment in which there are unwanted microorganisms. If the unwanted microorganisms are introduced into the colony of microorganisms with which we are working they are called contaminants. We cannot see them with our eyes so we must "see" them with our minds! In handling microorganisms we must visualize every way in which contaminants can get into cultures. Then we handle the cultures in such a way as to prevent the entrance of contaminants. Make a list of ways in which contaminants might get into a culture being transferred from one test tube to another and from one covered dish to another. (3) How can such contamination be prevented? (4)

Equipment

The types of equipment used in dealing with microorganisms are illustrated in Figure 9-1-1. The autoclave is used to sterilize all equipment and media not damaged by heat. Pipettes are of two types: calibrated glass tubes into which liquid can be sucked; and medicine-dropper pipettes provided with a rubber bulb at one end to give the necessary suction. *Caution must be observed when sucking up liquids by mouth into pipettes to take care that no liquid enters the mouth.* The Petri dish, named after one of Robert Koch's students, comes in two parts. Each part is a flat-bottomed glass dish with a small rim. A culture medium is poured into the smaller half of the dish; the other half serves as a lid to prevent microorganisms in the air from getting onto or into the culture medium. Any glassware used in microbiological experiments can be washed and autoclaved.

Microorganisms are so small that it is impossible to handle them individually. The inoculating loop and the inoculating needle are simple tools designed to handle *clusters* of microorganisms. Before using the loop or needle, sterilize them by passing their tips through a flame until they are red hot, then allow them to cool in the air before use. But the loop or tip must not be allowed to touch anything except the material to be handled.

Culture Media

In nature many kinds of microorganisms are found growing together but when we grow, or culture, microorganisms in the laboratory, we usually want to separate the different kinds and grow each kind or species as a pure culture. What are the advantages of growing mi-

croorganisms as pure cultures? (5) One of the big problems in culturing microorganisms is finding a substance on which they can grow. In the early days of microbiology nutrient broths, or soups, containing all the ingredients necessary for growth were used. If Pasteur were interested in the microorganisms involved in wine production, he would put a tiny drop of the wine in one of these culture solutions. Because the original drop would frequently contain more than one species of microorganism, it would be difficult for him to obtain a pure culture.

The problem of separating one species of microorganism from others so that it can be grown in a pure culture was solved in the laboratories of the German microbiologist, Robert Koch (1843–1910). Koch, as well as several other biologists, had discovered that a solid rather than a liquid culture medium could be used to isolate pure cultures of microorganisms. As is often the case in science, the discoveries of Koch and his group were based on the observations of another investigator.

In 1872, Johann Samuel Schroeter noticed that bacteria would grow on the surface of several materials, including that of a cut potato. Here the bacteria formed a large mass which was later called a colony. When these individual colonies were examined under a microscope, each was found to consist of great numbers of bacteria, but the most important thing was that all of the organisms in a single colony were of the same species. Apparently individual cells had landed on the surface of the potato and had then multiplied to millions. These colonies of millions of cells thus became visible to the naked eye. All the bacteria in a single colony were derived from one cell and hence each colony formed a pure culture.

Koch tried spreading dilute suspensions of several species of bacteria over a solid culture medium. The individual cells would stick at different locations on the surface, and the invisible single cells would start to multiply. Eventually, as Schroeter had noted, millions of cells resulted from each original cell that stuck in a particular location. Each colony turned out to be a pure culture.

Potato was not a satisfactory culture medium for a number of reasons, firstly, that it was not an adequate food for all species of microorganisms so that only a few kinds would actually grow on it. Secondly, it was not sterile, and contaminants from the knife or from the air would become attached to its surface and would also grow and form colonies. Thus, it
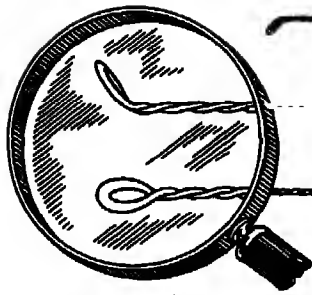
A. Inoculating loop

Welch Scientific Co.

B. Pipette

C. Inoculating needle
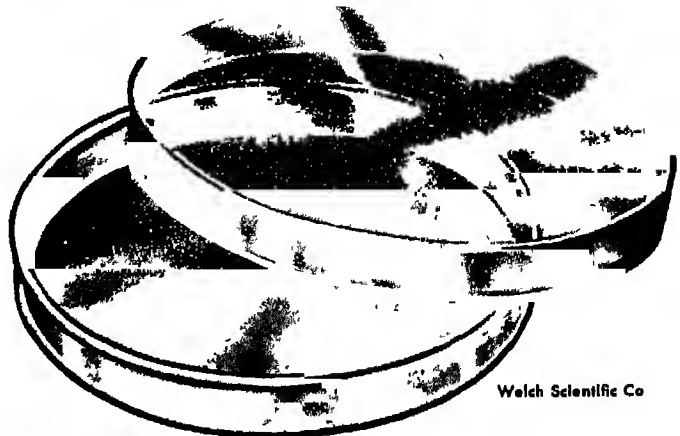
Welch Scientific Co.

D. Petri dishes

Welch Scientific Co

Aloe

Aloe

Welch Scientific Co.
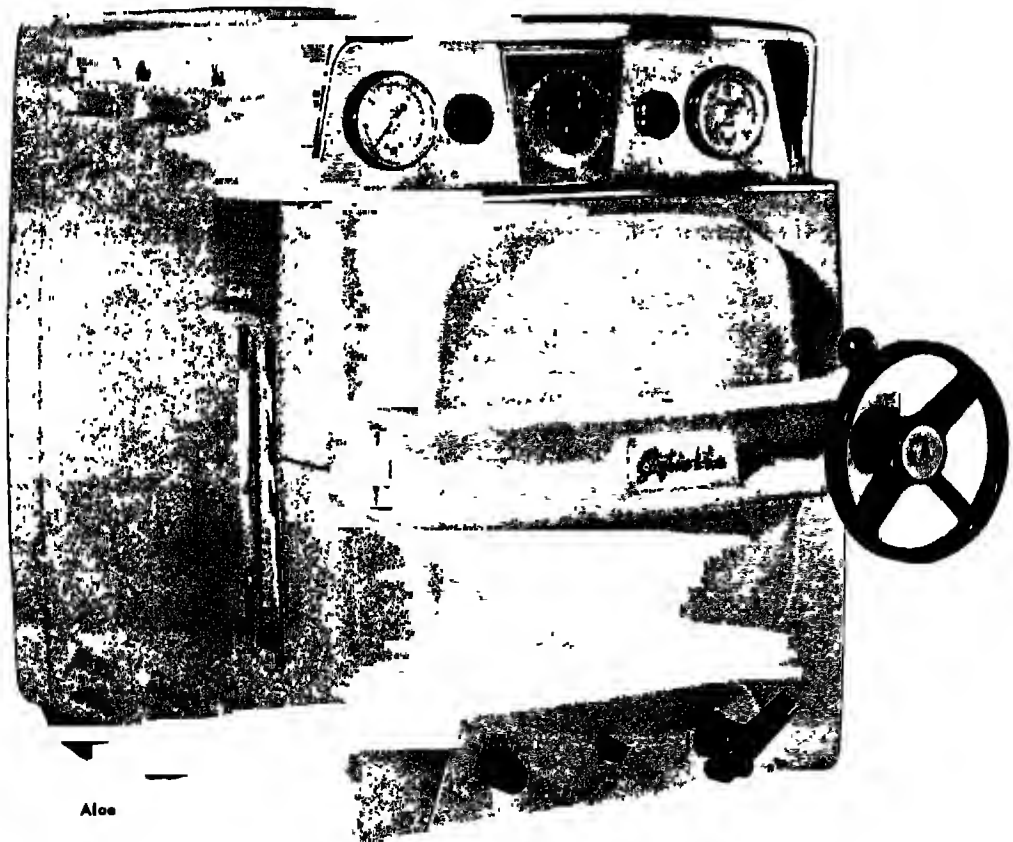
E. Autoclave

Figure 9-1-1   Types of microbiological equipment.

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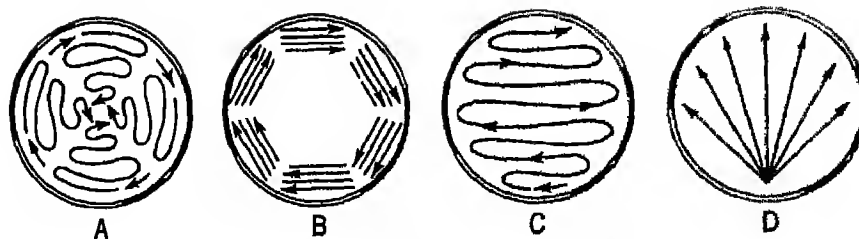
Figure 9-1-2   Streaking techniques.

was not always possible to be sure whether the colonies came from accidental contamination of the potato or from microorganisms purposely placed on the potato by the investigator. What was needed was a culture medium that would have all the substances necessary for growth of bacteria and also be sterile. Koch first used his nutrient broth or soup, which he mixed with gelatin to make it solid. The sterilized mixture was poured into Petri dishes to solidify. On this surface bacteria were spread, a process called streaking. (Figure 9-1-2 shows several different patterns used in the streak-plate method). Gelatin, too, has its disadvantages. It melts at 28° C, which is below the best temperature for the growth of many bacteria. In addition, many microorganisms make an enzyme, gelatinase, which digests gelatin and thus causes the medium to liquefy. Whenever the medium liquefies, the separate, pure colonies run together and mix.

A woman by the name of Hesse suggested that Koch try agar, an extract of seaweed. Agar proved to be the ideal medium for the solid culture technique. It is attacked only by a few microbial species and it does not soften until a temperature of 100° C is reached. The liquid agar does not then solidify until it is cooled to 42° C. The fact that it remains liquid until cooled to this low temperature made it possible to refine the methods of obtaining pure cultures. Thus, instead of using the streaking technique to isolate colonies of bacteria, one can mix the culture with the cooling agar while the agar is still liquid. The mixture can then be poured into sterile Petri dishes. When the agar solidifies the individual bacteria are found to be trapped at different locations in the agar where they develop into separate pure colonies. This technique of obtaining pure bacterial cultures is referred to as the pour-plate method. In the two photographs of Figure 9-1-3 below, the isolation of bacterial colonies both by the streak-plate method and by the pour-plate method may be seen and contrasted.

Today we still use the pure culture techniques of Koch in microbiological studies and then grow the microorganisms in an incubator at the proper temperature.
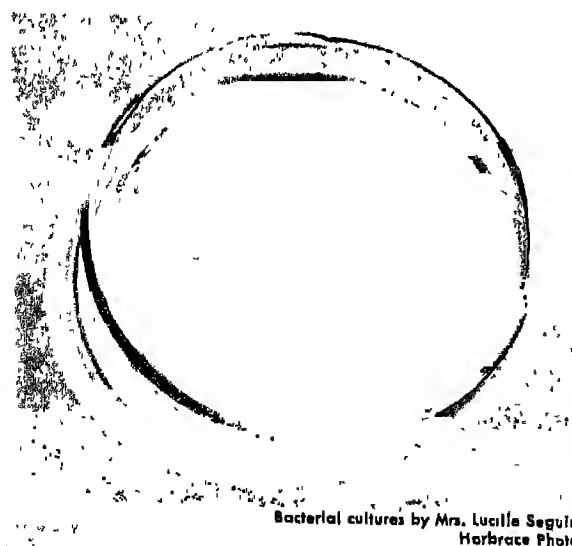
Bacterial cultures by Mrs. Lucille Seguin
Harbrace Photo
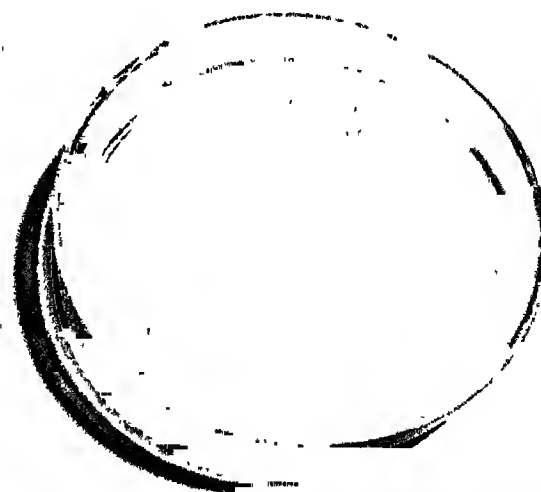
Bacterial cultures by Mrs. Lucille Seguin
Harbrace Photo

Figure 9-1-3   Streak plate (left) and pour plate (right) of Escherichia coli.

Prevention of Contamination

In any microbial culture the medium must be freed of foreign organisms first by sterilization, and second by providing barriers to the introduction of contaminants. The use of cotton to filter out airborne microorganisms has been practiced since 1855 when a pharmacist used a cotton plug to keep molds out of his drug preparations. Making media, preparing the culture containers with cotton plugs, and sterilizing both glassware and media are necessary preparations for growing microorganisms.

Staining Techniques

In order to see certain bacteria, staining techniques are used which are not greatly different from those practiced in earlier laboratory exercises to see nuclei and other parts of cells. The dye, crystal violet, reacts with the RNA of the cell surface and therefore those bacteria that contain a large amount of RNA in their surface layers are stained one color, those containing small amounts are stained a different color. This is the basis of a staining test developed by a Danish bacteriologist, Christian Gram. Depending on how the cells stain, the species of bacteria are classified as Gram-positive when they do stain, or Gram-negative when they do not stain. Because of the difficulty of classifying bacteria on the basis of structure, the **Gram staining technique** has been of great value in separating various types of bacteria.

Microscope Technique

Many times in order to study bacteria, it is necessary to use a higher power microscope objective than the 43 × on most student microscopes. For this purpose an **oil immersion objective** is used which gives a much higher resolution. However, in order to utilize this objective, it is necessary to place a drop of oil between the objective and the cover glass, the oil touching both.

Another frequently used technique is that of **dark-field microscopy**. This requires a special condenser which produces a black background against which microorganisms appear brightly illuminated.

Laboratory Technique

In handling microorganisms you must be careful not to distribute them carelessly about the working area. This precaution would be essential if microorganisms that cause disease were being used. However, even though you will not be working with disease-producing microorganisms you should learn to treat all cultures as if they were. Develop a steady hand for the careful transfer of microbial growth. Do not leave a trail of microorganisms behind you. Sterilize the inoculating loop and needle after you have used them and before putting them down. Put all cultures and other materials containing living microorganisms in containers to be sterilized. Use disinfectant solutions to clean work areas. All these practices are designed for everyone's welfare.

The BSCS high school biology technique film entitled "Bacteriological Techniques" may be shown. This film demonstrates some of the procedures about which you have been reading. Pay particular attention to these carefully demonstrated techniques.

Your teacher will demonstrate basic microbiological techniques and you may be allowed to practice transfers, streaking, media preparation, and other such techniques as time permits.

It is essential that you practice these techniques until you become skilled at working with microorganisms before you proceed to the microorganism exercises in this section.

On the basis of the above discussion, the film, the demonstration, and your own practice, answer the following questions. Why would it not be simpler and better to cork the test tubes or flasks rather than using cotton plugs? (6) A biologist transfers a microorganism from Petri dish A to Petri dish B. A week later he finds an entirely different microorganism in Petri dish B from that which he had transferred there. How could you account for this? (7) Supposing agar solidified at a temperature of 98° C instead of 42° C. Would it be a better or worse culture medium? (8) Why? (9) Suppose a test tube containing a culture of disease-producing microorganisms were to drop on a laboratory table. How would you go about sterilizing the area to remove all possibility of contamination? (10)

A GARDEN OF MICROORGANISMS

Microorganisms occur everywhere in the world—from the Arctic to the Antarctic, and from the depth of the oceans to the tops of the highest mountains. While they are not as abundant in some areas of the globe as they are in others, nonetheless it would be difficult indeed to find a microorganism-free environment occurring naturally on the face of the earth. Conditions favorable to the life of man are also favorable for microorganisms, and therefore microorganisms occur everywhere there are people, and in many other places. Many substances will serve for the growth of microorganisms.

■ The purpose of this exercise is to demonstrate the variety of environments that will support microbial growth, to learn how to distinguish common patterns of microbial growth, and to recognize colonies of microorganisms macroscopically (with the unaided eye), as well as to observe microorganisms under the microscope.

PROCEDURE

Number the culture dishes from 1 through 9 and add the following to each dish:

Dish 1—Rotting fruit
Dish 2—Slightly crushed grapes with sufficient water to cover

Dish 3—Enough hay to cover the bottom of the dish, and 500 ml water
Dish 4—A few dried beans and 500 ml water
Dish 5—Cottage or cream cheese spread over bottom of dish and 50 ml water
Dish 6—Quarter of lettuce head in about 10 ml water
Dish 7—Two pieces of stale or moldy bread, a small piece of squash or pumpkin to which some dust has been added, and just enough water to moisten the material before exposing to the air for 24 hours.
Dish 8—A piece of filter paper on the bottom of the culture dish. Mix 50 ml of aqueous 5% cornstarch with 350 g of rich soil. Cover the bottom of the dish with this mixture. Keep the soil moist.
Dish 9—20 g of peppercorns and 200 ml of water.

Place the dishes in a stack, except for Dish 1, and cover the top dish with a piece of glass. Do not place in direct sunlight. If the dishes fit too closely together, place a small piece of adhesive tape between each one.

Place Dish 1 a few yards away from the stack of dishes and cover it with a piece of glass. The organism which will develop here will form spores (asexual reproductive structures) quickly and may contaminate the rest of the dishes.

MATERIALS

Rotting fruits, such as citrus fruits, tomatoes, apples	Ten 11- or 20-cm glass culture dishes, or comparable clear plastic containers, pint jars, or deep Pyrex cake pans
Grapes	20 g of peppercorns
Moldy or stale bread	Squash or pumpkin
Cottage or cream cheese	Cornstarch
Hay, dried beans, and head lettuce	Compound microscope
Filter paper	Stereoscopic dissecting microscope
Rich soil	Adhesive tape
Wax pencil or gummed labels	Pipette
Two glass covers for culture dishes	
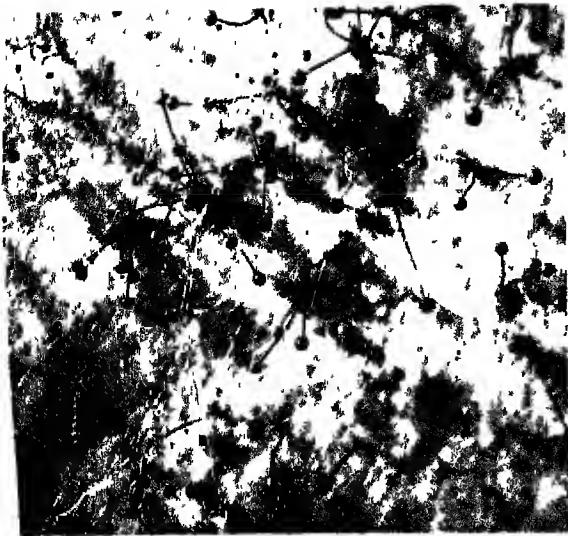
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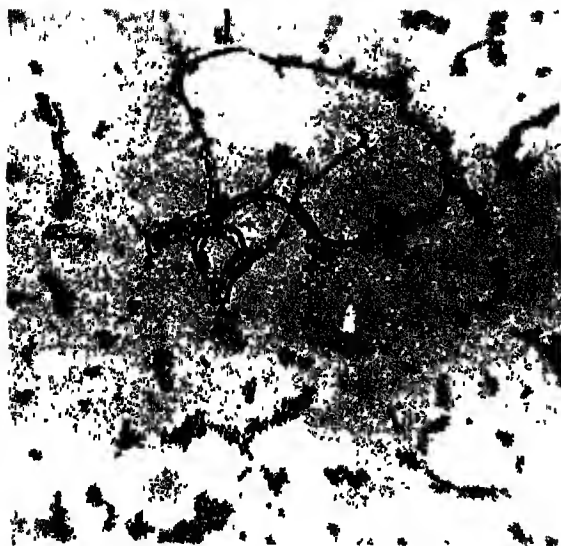
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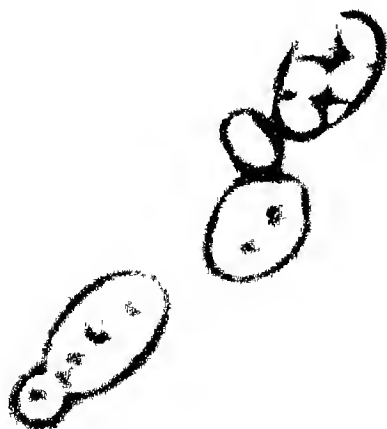
Dr. Carl C. Lindegren

Figure 9-2-1   A. Sporangia and mycelium of bread mold (*Rhizopus*); B. coccus (spherical) and bacillus (rod-shaped) bacteria; C. budding yeasts.

For each dish prepare a chart headed with the number of the dish and its contents. For each, describe carefully the condition of its contents as they appeared originally. Note particularly the extent of damage, color, and odor of the materials now in the dishes. For each day that observations are carried out, start a new paragraph headed with the date and continue to describe the contents of the dishes.

Based on your observations, answer the following specific questions. In which dish does microscopic growth appear first? (1) In which dish does it appear last? (2) In which of the dishes is there the greatest amount of growth of microorganisms? (3) In which of the dishes is there the least amount of growth of microorganisms? (4) In which of the dishes is the odor the strongest? (5) In which of the dishes is there the least odor? (6) Do all of the growths of microorganisms look the same in each dish? If not, describe in a short paragraph the different kinds of growths that appear. (7)

The main microbial types are molds, bacteria, yeasts, algae, and protozoans, some of which are illustrated in Figure 9-2-1. The algae and protozoans are illustrated in Figure 3-7-1 in Exercise 3-7. Which of these can be seen with the unaided eye? (8) How would you suspect the presence of those microorganisms which cannot be seen with the unaided eye? (9)

Take bits of the visible growths and mount them in water on slides for microscopic examination. Are all these samples of the growths similar in appearance? (10) Draw some of the typical growths under the microscope.

Examine drops of water taken from the dishes on different days. Try to identify the organisms seen as to whether they are molds, bacteria, yeasts, algae, or protozoans. Contrast the organisms found in this water with those seen earlier in Exercise 3-7. Are any of the organisms similar? (11)

Upon the completion of observations of the garden of microorganisms over a period of several days, make a statement regarding your conclusions as to where microorganisms may grow, the types of microorganisms you have seen, the rapidity with which the microorganisms have grown, and the results of microbial growth. Suggest ways in which growth of microorganisms can be hindered or prevented. (12)

Retain all cultures for further study. Be sure you place them in a suitable spot to favor their continued growth. They will be especially useful in Exercises 10-1, 10-3, and 11-3.

STAINING AND OBSERVING

BACTERIAL CELLS

In 1676 Antony van Leeuwenhoek, a Dutch lens grinder, mixed peppercorns (unground pepper) with water and after a week or two looked at a drop of this infusion with his simple microscope. In the infusion he discovered bacteria.

Today high-powered oil immersion microscopes are used to observe bacteria. With such instruments you can see bacteria magnified almost 1000 times. Living bacteria can be seen in wet mounts, but more often they are stained so that you can see them more easily.

■ The purpose of this exercise is to use a staining technique to reveal a few characteristics of bacteria grown in a peppercorn infusion.

MATERIALS

Peppercorn infusion from Exercise 9-2 (Dish 9)

Glass tumbler

Crystal violet stain

Paper towels

Compound microscope with oil immersion objective

Immersion oil

Inoculating loop

Microscope slide

Bunsen burner

Pipette

PROCEDURE
Technique for Staining Bacteria

There are three main parts to this technique, each with several steps as follows:

1. Preparing the bacterial film on the microscope slide:

a. Using a very clean slide, free of oily or greasy material, heat gently by passing it three times above the blue cone of a burner. Test the under surface of the slide on the inside of your wrist. It should be warm (not hot) to the touch.

b. When the slide is cool, place a small loop of peppercorn infusion on the slide and spread it thinly over an area the size of a dime.

c. Let the drop dry in the air to form a film on the slide.

2. Fixing the bacterial film onto the slide:

a. Quickly pass the slide three or four times through the flame, with the film side up, in order to fix or stick the bacteria onto the slide.

b. Let the slide cool to room temperature.

3. Staining the bacterial film:

a. Dip the slide into a glass of clean water.

b. While the film is wet, cover it with one or two drops of crystal violet.

c. Leave the dye on the film for 15 or 20 seconds before pouring off the excess.

d. Gently rinse the slide in a tumbler of clean water. Repeat the rinsing with clear water.

e. Drain the water from a corner of the slide.

f. Remove the remaining water with a piece of paper toweling by *gently blotting.* (CAUTION: *Do not rub the film.*)

g. When the film is dry it is ready to be examined microscopically.

Bacteria Under the Compound Microscope

Use the low-power objective to focus on the stained bacteria. Describe what you see. (1)

Now swing the high-power objective into place. If necessary, refocus with the fine adjustment. What greater detail is seen under high power? (2)

Bacteria Under the Oil Immersion Objective

Swing the high-power objective out of place. Put one drop of immersion oil directly onto the stained bacterial film. Swing the oil immersion objective into place. The tip of the objective will go into the drop of oil. The oil makes a continuous medium between the slide and the lens system of the objective.

You are now ready to focus the oil immersion objective on the stained bacteria. (CAUTION: *Focusing should be done with very great care to prevent breaking slides and damaging the objective.*) Use only the *fine adjustment* and turn it slowly to bring the colored film into clear focus. In what way do the bacteria you are observing differ from one another? (3)

Three general morphological types of bacteria exist. These are the spherical form, or cocous; the rod-shaped form, or bacillus; and the spiral forms, or spirilla, which may be of several different types.

The cocci may appear as single cells, as pairs of cells, as clusters of cells, or as chains of cells. Locate and draw several types of cocci as seen in your microscope field.

A rod-shaped bacillus may appear long and slender, or short and thick. Are bacilli found in chains or clusters, like the cocci? (4) Locate and draw several kinds of bacilli as seen under your microscope.

The spirilla are more difficult to see. If possible, locate and draw a spiral bacterium as seen under your microscope.

Which of the three types of bacteria you have observed appears to be largest? (5)

Determine the approximate dimensions of the different kinds of bacteria and record these sizes in microns.

The bacteria you are watching are harmless to man. With the use of your textbook and reference books determine what human diseases are caused by particular species of cocci, bacilli, or spirilla. (6)

DISTRIBUTION

OF MICROORGANISMS

Making microorganism imprints is one of the easiest ways to find out how widely microorganisms are distributed in our immediate environment. The technique is especially useful for detecting microorganisms on solid materials. It can be done by using a piece of cellophane tape to transfer microorganisms from a surface to an agar plate.

■ The purpose of this exercise is to demonstrate the presence of microorganisms in our surrounding environment and to suggest means of reducing microorganism populations on surfaces.

MATERIALS

Roll of cellophane tape, 1 cm (½ inch) wide
One sterile Petri dish containing sterile nutrient agar
Water bath
Thermometer
Wax pencil
125-ml flask

PROCEDURE

Before class: Prepare the media and sterilize the Petri dishes before the day of the experiment.

Laboratory period: On the *bottom* of the Petri dish containing nutrient agar draw, with a wax pencil, two intersecting lines that divide the dish into quarters. Number the quarters 1 through 4.

Pull a 10-cm strip of cellophane tape from the roll.

Fold over the end of the cellophane tape for about 2 cm in order to provide a nonsticky end for holding the tape.

Holding the tape at the folded end, put the sticky side on any solid surface in the classroom, laboratory, lavatory, cafeteria, water fountain, shower room floor, or food preparation surfaces in the home economics laboratory. Cooking and eating utensils may also be used. Be sure to test some cleaned surfaces of various kinds as well as dirty surfaces.
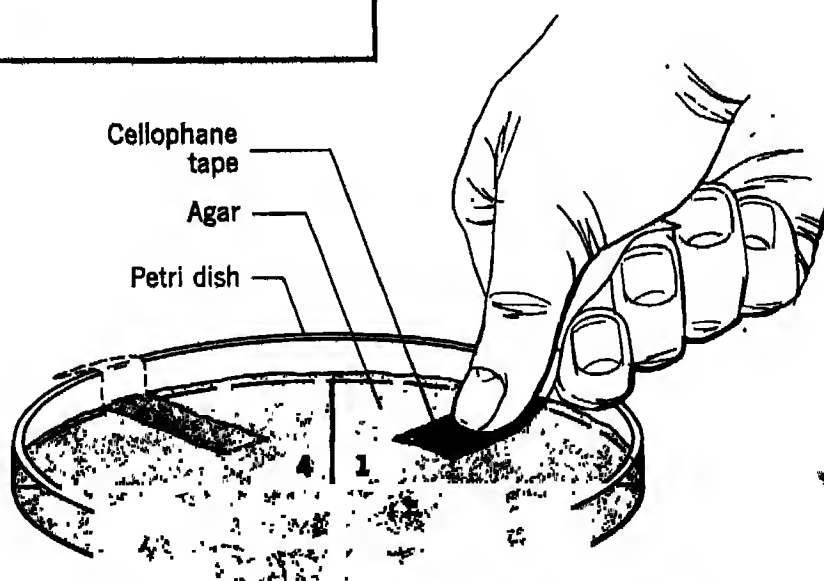
Figure 10-2-1 Cellophane-tape technique on agar plate.

Pull the tape from the surface, and immediately place it on the agar surface of the number 1 quarter of the dish (Figure 10-2-1). If necessary, gently press the tape onto the agar to transfer the microorganisms. Then remove and discard the tape.

With fresh strips of cellophane tape, repeat for two additional surfaces, placing the strips on quarters 2 and 3. As a control, repeat with a fourth strip of tape that has not been touched to a surface.

Record, in chart form, the source of the microorganisms for each of the four quarters.

Incubate the plates at room temperature for three or four days. Observe them daily, and record the results on the chart.

On the blackboard make a master chart of the kind and number of microorganisms found on all the different surfaces examined by the class.

Were any examined surfaces free of microorganisms? (1) How do you account for this? (2)

Which were more abundant, molds or bacteria? (3)

How might the microorganisms of disease be spread by the medium of surfaces? (4) How can this possibility be reduced? (5)

PURE CULTURES

OF MICROORGANISMS

The need for pure cultures of bacteria was especially evident to Robert Koch when he studied infectious diseases. The problem was to determine which bacterium, of the many associated with the diseased animal or plant and its environment, causes a given disease. To do this would require the separation of the different kinds of microorganisms present.

Since microorganisms are so small and so mixed together in their natural habitats, the separation of different kinds cannot be done as easily as one can separate dandelions from grass in a lawn. Yet it is possible to make this separation and to obtain pure cultures—cultures containing only one kind of microorganism.

How are microorganisms separated? You will use the techniques that Koch and his coworkers developed and that are still used today to isolate pure cultures of all types of microorganisms.

■ The purpose of this exercise is to demonstrate the technique of dilution for obtaining pure cultures of microorganisms by two methods: the pour-plate method and the streak-plate method.

PROCEDURE (first day)
Preparation of Culture Medium
(each 10 students)

Mark the 15-ml level on 25 clean test tubes as follows: place 15 ml of water in one tube and mark the water level with a wax pencil. Pour out the water and use this tube as a measure to mark the 15-ml line with wax pencil on the other 24 tubes.

Fill all 25 tubes to the 15-ml mark with nutrient agar medium or beef-bouillon agar medium and plug with cotton.

Using a 10-ml pipette, add 9 ml of distilled water to each of 15 additional clean test tubes and plug with cotton.

Sterilize all 40 tubes in an autoclave or pressure cooker at 15 lbs pressure for 15 minutes (121.5° C). Each group of students should have three tubes of water and five tubes of agar.

PROCEDURE (second day)
Preparation of Plate Cultures

Each group of students will use one of the following sources of bacteria:

| Soil suspension | Pepper water | S. marcescens |
| Hay infusion | Stagnant pond water | and S. lutea mixture |

MATERIALS

400 ml nutrient agar or beef-bouillon agar	Hay infusion
Two wire test-tube baskets	Mixed suspension of *Serratia marcescens* and *Sarcina lutea*
Water bath (double boiler)	
Two to five inoculating loops	Pepper water
One 1-liter flask	Stagnant pond water
Forty test tubes, 150 x 18 mm	Wax pencil
Twenty-five Petri dishes	Cotton plugs
One 500-ml graduated cylinder	Bunsen burner
Five 10-ml pipettes	Towel
Soil suspension (1 g garden soil in 5 ml water)	Paper marked in 1-cm grid
	Centimeter ruler
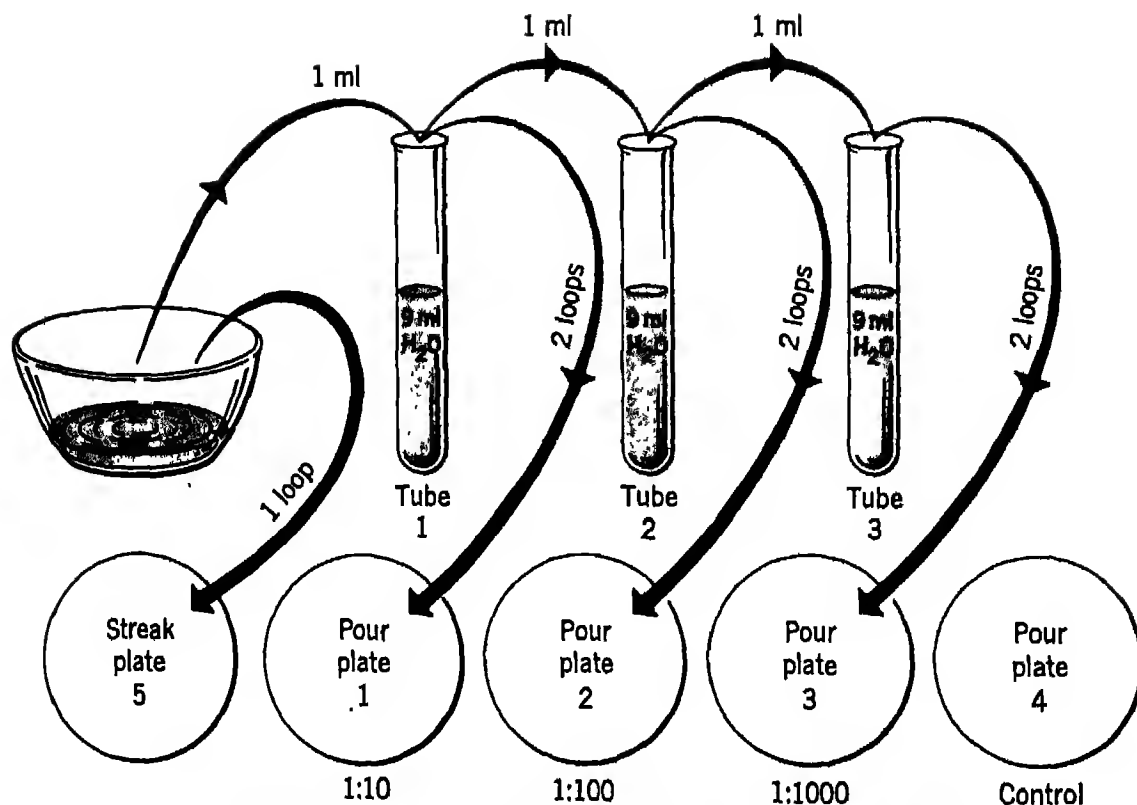
Figure 10-3-1 Method of dilution and inoculation of pour plates.

Number the three test tubes of sterile water 1, 2, and 3 and place them in a rack. Number five sterile Petri dishes 1 through 5.

Set up a boiling-water bath to melt the agar in the tubes. While agar is melting make dilutions for the following.

I. Pour-Plate Method

1. With a sterile pipette add 1 ml of source material to Tube 1. Mix thoroughly by gentle tapping or rolling the tube between the palms of the hands. What is the dilution of the source material in Tube 1? (1)

2. As above, transfer 1 ml of the fluid in Tube 1 to Tube 2. Mix as above. What is the dilution of the source material in Tube 2? (2) How many times more dilute is the solution in Tube 2 than in Tube 1? (3)

3. As in step 1 above, transfer 1 ml of the fluid in Tube 2 to Tube 3. Mix as before. What is the dilution of the source material in Tube 3? (4) How many times more dilute is the solution in Tube 3 than in Tube 2? (5) Than in Tube 1? (6)

If, instead of using 1 ml of material, two loops (0.1 ml) had been transferred each time, would the same dilutions be obtained? (7) Would the ratio of dilution between the tubes be the same as that of the 1.0 ml transfers described above? (8)

4. Sterilize a bacteriological loop in flame, cool, and transfer two loops of material (approximately 0.1 ml) from Tube 1 to Plate 1, spreading the liquid over the bottom of the plate. Be sure and keep the plate covered as much as possible during the transfer, and cover it immediately after the transfer. Remember to flame the loop and tube lip *before* and *after* each transfer. Replace the cotton plugs in the tubes immediately. Do not put the plugs on the disk but hold them. Review the techniques of Exercise 9-1 if necessary.

5. As above, transfer two loops from Tube 2 to Plate 2.

6. As above, transfer two loops from Tube 3 to Plate 3.

When the agar in the five tubes has melted, cool in the water bath to 45° C, and pour one tube of agar into each numbered plate. Dry off the tube with a towel and flame the mouth of the tube before pouring the plates. Do not allow the tube lip to touch the inside of the plate. Plate 4 serves as a control, containing agar but no source of microorganisms.

II. Streak-Plate Method

When the agar of Plate 5 has solidified, flame the loop, cool, take a loop of one of the original source materials, and streak the surface of the agar. *Do not dig or cut into the agar.*

Use one of the methods illustrated in Figure 9-1-2 to streak the plate.

Incubation of Plates. When the agar in the plates has solidified, incubate them upside down at room temperature. (Do not incubate the plates near a radiator or in direct sunlight. 25° C is a good temperature.) Observe daily for three days, after which time final observations are to be recorded.

Place each Petri dish over a paper divided into 1 cm squares. Count the number of colonies per square cm and record on the Bacterial Colonies Chart on page 86 of this exercise. Which source has given the greatest number of colonies *per plate?* (9) The least? (10) What has been the effect of dilution on each of the sources? (11) Would it be possible to get colonies if further dilutions were carried out to 1 : 1,000,000? (12) If you wanted to dilute further, which sources would be logical to dilute to this extent? (13)

On the Chart of Observations on this page or on a similar chart, diagram the colonies on the streak plate and on a segment of each of the pour plates, as indicated. Which method produced the greatest number of colonies? (14) Which is most effective in obtaining pure cultures? (15)

Assume that each bacterial colony has arisen from a single bacterial cell. How many cells were transferred to each plate from each of the tubes? (16) How many cells were in each milliliter of the tubes? (17) How many cells were in each milliliter of the source material? (18)

Did any colonies develop in the control plate? (19) What does the state of the control tell you about the technique you used in this experiment? (20)

CHART OF OBSERVATIONS
Plates Inoculated with _____
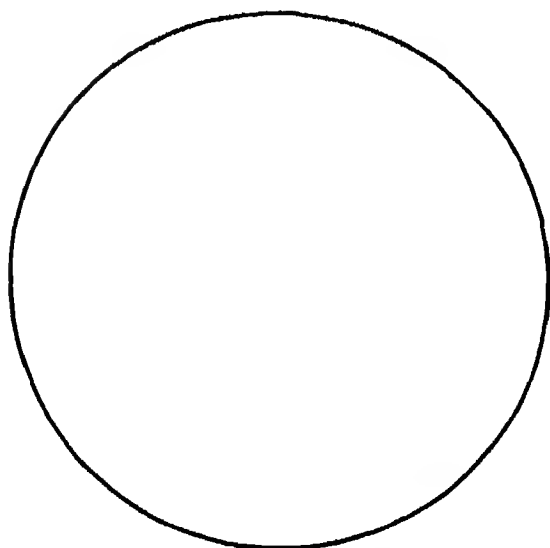
Colonies on Agar Streak Plate
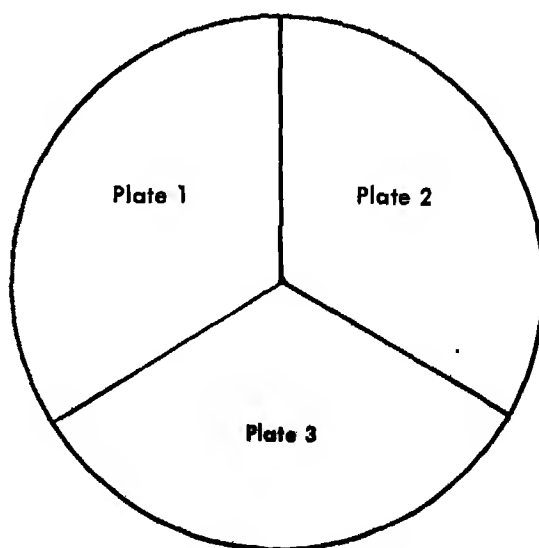
Composite Colonies
on Dilution Agar Pour Plate

(The three sectors represent segments of Plates 1, 2, and 3.)

BACTERIAL COLONIES CHART

Material	Number of Bacterial Colonies in 1 square cm			
	Pour Plate 1	Pour Plate 2	Pour Plate 3	Streak Plate
Soil suspension				
Hay infusion				
Pepper water				
Stagnant pond water				
Serratia marcescens and Sarcina lutea				

ANTIBIOTICS AND BACTERIA

You have heard of antibiotics and you probably have taken some of them for various illnesses. In this exercise we will use disks which have been soaked in antibiotics and will place them on agar plates containing bacteria. We will then observe the results.

■ The purpose of this exercise is to determine the effects of various antibiotics upon different bacteria.

MATERIALS

Commercial antibiotic disks, containing
    agents such as penicillin, streptomycin,
    tetracycline, erythromycin, chloramphen-
    icol, neomycin, or cholistin
Broth cultures of *Staphylococcus albus* and
    Escherichia coli
Nutrient agar plates, two for each team of
    students
Wax pencil
Forceps
Inoculating loop
Millimeter ruler
Bunsen burner

PROCEDURE

A procedure similar to this one is used in hospital diagnostic laboratories to determine what chemical or antibiotic should be used to treat a particular disease.

Divide each nutrient agar plate into three sections and number them 1, 2, and 3 by marking on the bottom of the dish with a wax pencil.

Streak the entire surface of Plate 1 with *S. albus*.

Streak the entire surface of Plate 2 with *E. coli*.

Use forceps to lay a different antibiotic disk on the surface of each section of Plate 1. Use the same kinds of antibiotic disks on Plate 2. Be sure the disks are firmly stuck to the agar surface.

Invert and incubate at 37° C.

Observe after 1 or 2 days for clear areas around the disks, which show the extent to which microbial growth has been inhibited.

Measure the diameter of the clear areas around each disk and record your observations.

Which of the two organisms is more sensitive to antibiotics in general? (1) Are both organisms equally sensitive to each of the antibiotics used? (2) Which antibiotic would you use to control *S. albus*? (3) To control *E. coli*? (4) *E. coli* is normally present in the human intestines. Does the sensitivity of *E. coli* to the antibiotics suggest to you one reason why doctors advise that antibiotics be used only when necessary? (5) What does this experiment tell you about antibiotics? (6)

Can you be sure that the inhibition of microorganisms is due to the antibiotics on the disks? (7) What feature does this experiment lack which it should have? (8) How would you correct this omission? (9)

TEMPERATURE

AND MICROORGANISMS

Microorganisms are literally at the mercy of their environment and may be affected favorably or unfavorably by very slight changes in it. An organism can exist only within definite and narrow limits of the environment.

Among the more important environmental conditions affecting microbial growth are temperature, moisture, oxygen, food supply, hydrogen ion concentration, the presence of certain chemicals, and the presence of other organisms.

Temperature, which we will study here, is important since all growth processes depend on chemical reactions whose rates temperature influences. Temperature can determine the rate and amount of growth and the metabolism and morphology of the microorganism.

■ The purpose of this exercise is to help you become more familiar with one of the environmental factors mentioned above, temperature, and its effect on microorganisms. We will also see how man uses this knowledge to control microorganisms.

MATERIALS

Twelve sterile plugged test tubes

**Raw skim milk (or, if not available, 99 parts
    pasteurized skim milk to which 1 part
    buttermilk has been added)**

Refrigerator

Incubator

Water bath

Slides

Compound microscope

Crystal violet stain

Wax pencil

Graduated cylinder

Four test-tube racks

PROCEDURE

This should be done in groups of three students, each student performing one part.

1. To each of four clean sterile test tubes add 30 ml of raw skim milk. Number the tubes 1, 2, 3, and 4 with your wax pencil. Store Tube 1 in a rack in the refrigerator at about 7° C, Tube 2 in a rack at room temperature (about 23° C), Tube 3 in a rack in an incubator at 37° C, and Tube 4 in an incubator (if available) at about 45° C.

2. Fill four additional sterile test tubes each with 30 ml of the milk. Heat the tubes at 62° C in a water bath for 30 minutes. Number the tubes 1 through 4, and incubate each tube as you did in Procedure 1.

3. Fill four more sterile test tubes with the milk as you did above, and heat the tubes at 90° C for 10 minutes in a water bath. Number each tube as above and incubate each tube as directed under Procedure 1.

Observe the tubes of milk daily for a week. Make a chart showing treatment of milk, temperature of incubation, and time of change in appearance in days. Record the changes in the appearance of the milk. Record the number of days required for the changes to appear.

Examine samples of milk on successive days by placing a drop of the milk on a slide. Stain the slides as in Exercise 10-1. What microorganisms are present? (1)

In what tubes are microorganisms most abundant? (2)

Why is heating of milk (pasteurization) an approved milk-processing technique? (3).

Under what temperatures should milk be stored to reduce growth of microorganisms? (4)

At what temperatures are microorganisms killed by the techniques of this exercise? (5)

What general statement can be made about the effect of temperature on microbial growth in milk? (6)

DIGESTION OF FOODS
BY MICROORGANISMS

The microorganisms we most often en-counter are those that use a great variety of foods in their nutrition. Most of these foods are organic substances such as proteins, car-bohydrates, and fats. Since these substances are complex molecules, they cannot usually be taken into cells until they are broken down into simpler compounds which pass through the cell wall and cell membrane into the cell where they are then used in metabolism.

Microorganisms vary greatly in their ability to break down or digest the complex carbo-hydrates. Some decompose cellulose; some act on starch and glycogen; a large number attack disaccharides such as cane sugar (sucrose), malt sugar (maltose), or milk sugar (lactose). The digestive enzymes are synthesized within the cell and secreted into the environment of the cell; there they carry on digestion.

Microorganisms also vary in their abilities to digest different proteins such as gelatin, plant proteins, or casein in milk.

■ The purpose of this exercise is to dem-onstrate how common microorganisms digest a complex carbohydrate (starch) and a protein (casein) and to compare their nutritional re-quirements.

Part A:
Starch Digestion

PROCEDURE

1. Melt the nutrient starch agar and pour 15–20 ml into each sterile dish.
2. When the agar has solidified, divide one dish into two sections by means of a line drawn with a wax pencil on the bottom of the dish. Label one section HAY, the other YEAST.
3. Use a sterile loop to make on the hay sec-tion a single streak from the hay infusion.
4. Streak the yeast section with a single streak of the yeast suspension.
5. Streak the second dish with a single streak of the mold suspension. Label it MOLD.
6. Divide and streak two control dishes made with nutrient (no starch) agar as in 3, 4, and 5 above.
7. Invert these dishes and incubate them at room temperature until good growth is evident (2–4 days).
8. After growth is evident, cover the surface of all four dishes with dilute iodine solution. After about 30 seconds pour it off. The inter-action of starch and iodine produces a blue color.

MATERIALS

Part A
Sterile Petri dishes (four per student team)
400 ml sterile nutrient agar containing 0.2% starch
400 ml sterile nutrient agar
Iodine solution
Hay infusion
Millimeter ruler
Yeast suspension (few grains of dry yeast in water with enough syrup added to color slightly)

Mold suspension
Wax pencil
Inoculating loop
Bunsen burner

Part B
Skim milk
Hay infusion or *B. subtilis* culture
Two tubes of nutrient agar
Two sterile Petri dishes
Inoculating loop
Bunsen burner

9. Make a drawing of the starch agar dishes to show their appearance.

Is there a clear area around the streaks of hay, yeast, and mold? (1) Measure and record the extent of each clear area, if any. What does the presence of a clear area indicate? (2) What does its absence indicate? (3) Make a statement about starch digestion by the three microorganisms used. (4) What is the condition of the control dishes? (5) Compare the growth of microorganisms on starch and nonstarch media. Did the microorganisms do equally well on both types of plates? (6) Which did best on the nonstarch plates? (7)

Part B:

Protein Digestion

PROCEDURE

Add 1.0 ml of skim milk and one tube of molten nutrient agar (at 45° C) to a Petri dish. Mix and allow this milk-nutrient agar to solidify.

Make a single streak with a loopful of hay infusion or *B. subtilis* on the milk-nutrient agar surface.

Add one tube of nutrient agar to a Petri dish (without skim milk), streak as above, and label it CONTROL.

Incubate the two dishes at room temperature until good growth is evident (2–4 days).

Observe the dishes daily and notice the clear areas of the agar, a sign of protein digestion.

Make a drawing of the milk agar dish to show the area of protein digestion. Measure and record the extent of the clear areas.

What can you conclude about the ability of the microorganism to digest casein (the milk protein)? (8)

Compare the digestion of protein and starch. Which was more extensive? (9) Which took place more rapidly? (10)

This extracellular digestion reduces proteins to amino acids and reduces starches to simple sugars which then pass into the cell to be used as energy sources.

BACTERIAL POPULATIONS

IN MILK

Milk is secreted as a sterile fluid into ducts of the udder in a healthy cow, but it becomes contaminated as it passes through the milk ducts, which have a characteristic bacterial flora of harmless cocci and small bacilli. Moreover, unless great care is used during the milking process, a great variety of bacteria will enter the milk after it leaves the udder. Because milk is the "most nearly perfect food" for man, it is also an excellent medium for the nutrition and growth of bacteria and, therefore, care must be used in its processing.

If the milk is cooled immediately after milking and kept cold, only a slow increase in the number of bacteria will occur. The process of pasteurization will kill all harmful bacteria and many of the harmless ones. (Refer to Exercise 11-2.)

Actively growing bacteria in milk consume oxygen and bring about a lowered oxygen content. This can be detected by using methylene blue, which turns to a colorless compound in the absence of oxygen. When the number of bacteria in the milk is high, the rate of decolorization of the methylene blue dye is rapid; but if the numbers are low, a longer period will be required to decolor the methylene blue.

The following table shows how milk is rated according to results of the methylene blue test.

Decolorizing Time	Rate
More than 8 hours	Excellent
5½ to 8 hours	Good
2 to 5½ hours	Fair
20 minutes to 2 hours	Poor
Less than 20 minutes	Badly contaminated

■ The purpose of this exercise is to demonstrate the presence and amount of the bacteria contained in milk of various kinds.

MATERIALS

Methylene blue solution 1 : 30,000 or methylene blue thiocyanate tablets

Old and fresh samples of raw and pasteurized milk

Four test tubes, vials, or bottles for each team of students

Graduated cylinder

Water bath

PROCEDURE

Obtain four samples of milk of various ages and from different sources.

To each 10-ml sample of milk, in a test tube, add 1 ml of methylene blue solution.

Incubate the samples in a water bath at 37° C. Notice the time required to decolorize the methylene blue.

Rate the bacterial content of the milk samples you have tested. (1)

What is the relationship between your results and the age of the milk samples? (2)

List possible factors that would give a short decolorization time. Consider all contaminating factors operating from the time the milk leaves the cow to the time of consumption. (3)

What steps should be taken to lessen the effect of those factors? (4)

What are your conclusions regarding the effect of storage temperatures on the rate of multiplication of bacteria in milk? (5)

What factors could account for a high bacterial count in pasteurized milk? (6)

SUGAR FERMENTATION

BY YEASTS AND BACTERIA

Sugar is one of the principal sources of energy for microorganisms. The energy is used to regenerate ATP, the storehouse of energy in living cells.

When sugar is broken down to yield its energy in the presence of oxygen (aerobic conditions), carbon dioxide and water are the end products. If less oxygen is available, some microorganisms can obtain energy, although less efficiently, by fermentation. The process is inefficient since much energy still remains locked within the end products of fermentation, such as organic acids and alcohols.

Microorganisms differ in their ability to ferment the different sugars. In this experiment, the fermentation of glucose and lactose by bacteria and by yeast will be studied. In the fermentation tube the microorganisms are using oxygen more rapidly than it can diffuse into the broth so that they are creating an oxygen-deficient environment by their metabolism.

■ The purpose of this exercise is to compare and contrast the fermentation of different sugars by yeasts and by bacteria, and to draw conclusions regarding anaerobic metabolism.

MATERIALS

Thirty-two Durham fermentation tubes

297 ml of nutrient broth with 3 ml glucose added (300 ml)

250 ml of bromthymol blue indicator

1% lactose nutrient broth

Yeast suspension (Saccharomyces cerevisiae) or a few grains of dry yeast

Broth culture of bacteria (Escherichia coli, Staphylococcus albus, or soil suspension may be used.)

Eleven 1-ml pipettes

Millimeter ruler

Autoclave

PROCEDURES

Teams of eight students, each student with one fermentation tube, will do the experiment.

To each fermentation tube add 0.3 ml of bromthymol blue solution. To four of the fermentation tubes add enough glucose broth to cover the gas collection tube by 1 cm. To the other four fermentation tubes add enough lactose broth to cover the gas collection tube by 1 cm. Plug both sets of tubes and autoclave them at 15 lbs for 12 minutes. When the fermentation tubes are cool, make the following inoculations.

Inoculate one tube of lactose and one tube of glucose with the suspension of the yeast *Saccharomyces cerevisiae*, and label it. Inoculate one tube of lactose and one tube of glucose with *Escherichia coli* and label. Inoculate another tube of lactose and another tube of glucose with *Staphylococcus albus* and label. Do not inoculate the last tubes (one each of glucose and lactose). They are controls. Incubate all the tubes at room temperature. Make daily observations for 2 or 3 days. On a chart, record the changes in color of the indicator and the amount of gas in millimeters collected in each of the eight tubes. Make a bar graph showing the millimeters of gas produced in each of the eight tubes.

From what you know of metabolism, what might the gas in the tubes be? (1) How could you test for that kind of gas? (2) What kinds of metabolism produce this kind of gas? (3) What does the color change indicate? (4) What could have caused this color change? (5) Would this color change be characteristic of aerobic or anaerobic metabolism? (6) Why? (7)

In which tubes did fermentation occur most rapidly? (8) Least rapidly? (9) Which organism is the best anaerobe? (10) Do the microorganisms ferment better in glucose or lactose? (11) What does the condition of the control tell you about the technique of this experiment? (12)

Plants

Until a person takes an interest in plants, these living things tend to be just masses of green. We may recognize certain large plants as trees, complain about the weeds on our neighbor's lawn, and perhaps recognize a mushroom growing in the woods. But the recognition of trees, weeds, grasses, and mushrooms is just a small beginning in distinguishing approximately 350,000 different kinds of plants living in the world today.

Let us suppose that you have an overwhelming ambition to look at one specimen of each of these plants. One thing that would quickly impress you, if you actually began such a study, is the tremendous variety of plants living on the earth today. There would be plants having parts of almost every imaginable color. Some plants could be studied adequately only with a high-powered microscope. Others, such as large trees, would require climbing irons and scaffolding if they were to be inspected without cutting them down. There would be aquatic plants which swim from place to place and still other plants which resemble spots of paint adhering to sunbaked rocks.

How, in a few brief exercises, can we present an even moderately adequate picture of this world of living plants? To work with even a small proportion of them in detail would be a tremendous undertaking. Therefore we are going to try to decipher, in a general kind of way, the story of plant function and how the great variety derived by evolution from what was originally probably a very limited number of simple types.

It is from simple organisms that plants with more complex structures and plants capable of growing in a wide variety of land environments have evolved. Clearly, to understand plant evolution we must have some idea of what these ancestors were like as well as knowledge of the more familiar green plants of today.

Without green photosynthetic plants, you and I and all other living things would perish because our source of energy and of oxygen would soon be exhausted. While a plant is photosynthesizing water molecules are split and oxygen is released into the atmosphere. The plant absorbs and stores solar energy that either directly or indirectly is used as a source of energy by all forms of life.

Keep this in mind the next time you eat a beefsteak. Remember that the energy stored in the steak which you will digest and use was originally solar energy that was fixed by the green cells of the grass, alfalfa, corn, and other plants eaten by the steer. Do not forget that plants themselves require a share of solar energy for growth. When you eat a vegetable salad, broccoli heads, or sauerkraut, you, like the steer, are using the stored energy in the green plant—energy that was stored in the plant for its own growth and reproduction. *Remember plants do not grow for animals to eat!*

COMPARISON OF PLANTS—

SIMPLE OR COMPLEX?

The score card provided in this exercise is designed to emphasize a few characteristics which can be used to compare and contrast two or more kinds of plants regardless of their size or complexity.

The materials list suggests where the plants can be found in case the exercise is done during winter, when it is difficult to obtain some types of plants. Lichens and conifers can be collected in the field at any time of year.

■ The purpose of this exercise is to acquaint you with the great variety of plants which exist and with some of the features which serve to distinguish them from one another.

MATERIALS

Compound microscope
Dissecting microscope or hand lens
Various plants from the grocery store, local
    greenhouse, aquaria, homes, and from
    the field, containing reproductive as well
    as vegetative parts:
        Mushrooms (grocery)
        Lichen (tree bark or rocks)
        Algae (aquarium)
        Moss (greenhouse or terrarium)
        Fern (local florist or terrarium)
        Conifer (cone-bearing branch from a
            pine, spruce, or fir tree)
        Flowering plant (with flowers from
            local florist)

PROCEDURE

You are to "score" the plants with the chart (Figure 12-1-1). To determine the score of a particular plant, start at the box at the top marked START HERE. Lines from this point lead to two choices. Choose the one which fits the plant being scored. Each description on the page, except the last in a line, is followed by two lines which lead to two choices. In each case choose the description that best fits the plant being scored, and continue to the end of the line. The total score is the sum of all the numbers appearing in front of the choices used in your journey through the scoring key. In the Plant Scoring Chart at the end of this exercise, record the name of each plant, the numerical values of the choices made in scoring it, and its total score.

The more alike two plants are, the more alike their scores will be. The greater the differences between two plants, the greater the differences in their scores will be. Highly developed plants will have high scores (maximum, 26), while simple or primitive plants will have relatively low scores (minimum, 3).

Compare four or five different kinds of plants, using the score card. Now arrange these plants in order from the lowest scores to the highest. Notice the important characteristics of the low-scoring plants and those of the higher-scoring plants.

Assuming that most plants have developed from simpler, fewer, and older species, would you expect plants to show less or greater variety as time goes on? Explain. (1)

Basing your conclusions on your use of the plant score card, what do you consider to be some of the major (more important) differences among plants? (2) What are some of the less important differences? (3) On what basis do you distinguish between important differences and less important differences? (4)

In some instances the method of reproduction by spores or seeds will not be clear. Also, the distinction between roots and rhizoids may need explanation. Ask your teacher for help if you have difficulty. Make free use of the microscope and hand lens to see the details.

START HERE

(4) Plants with structures which look like roots, stems, and leaves (see next page)

(1) Plants without structures which look like roots, stems, or leaves

(2) Green, blue-green, or gray-green (with chlorophyll)

(1) Not green; usually white, gray, brown, or yellow (saprophytes or parasites)

(3) Crustlike or resembling gray, wilted leaves, flat-growing or upright and branching

(2) Usually aquatic, not flattened or leaflike

(2) Not microscopic in size

(1) Microscopic in size; single-celled; occurring singly or in chains

(5) Bright green; generally on damp soil

(4) Gray-green; generally occurring on rocks or tree trunks

(3) Umbrellalike, shelflike, or spherical; usually from 1 to 15 cm high

(2) Hairlike, slender, usually fuzzy or powdery, and less than 1 cm high

(3) Blue-green; chlorophyll diffused throughout cells, not in distinct bodies

(4) Grass-green; chlorophyll in definite bodies only

Figure 12-1-1   Plant scoring key.

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                        ┌─────────────────┐
                        │   START HERE    │
                        └─────────────────┘

┌──────────────────────────┐              ┌──────────────────────────────┐
│ (4) Plants with structures│              │ (1) Plants without structures which │
│     which look like roots, │              │     look like roots, stems, or leaves │
│     stems, and leaves      │              │     (see preceding page)      │
└──────────────────────────┘              └──────────────────────────────┘

┌──────────────────────────┐              ┌──────────────────────────────┐
│ (4) Roots, stems, and leaves│            │ (4) No true roots, stems, or leaves; │
│     present; usually more  │            │     hairlike rhizoids instead of │
│     than 10 cm tall        │            │     roots; not more than 10 cm tall │
└──────────────────────────┘              └──────────────────────────────┘

┌────────────────────┐  ┌──────────┐      ┌────────────────────┐  ┌────────────────────┐
│ (6) No seeds; reproduction│ (5) Produce│   │ (4) Leaflike structures in│ (5) Leaflike structures │
│     by spores      │  │     seeds │      │     two rows; without   │     spaced equally around │
└────────────────────┘  └──────────┘      │     midribs        │     stalk; with midribs │
                                          └────────────────────┘  └────────────────────┘

┌──────────────────────────┐              ┌────────────────────┐
│ (5) No flowers; seeds borne in cones;│  │ (5) Flowers present │
│     leaves usually needlelike │       └────────────────────┘
└──────────────────────────┘

┌────────────┐ ┌────────────┐   ┌────────────────────┐  ┌────────────────────┐
│ (1) Trees  │ │ (2) Shrubs │   │ (4) Flower parts in four's or│ (4) Flower parts in three's; │
└────────────┘ └────────────┘   │     five's; leaf veins netted│     leaf veins parallel │
                                └────────────────────┘  └────────────────────┘

┌──────────────────┐ ┌──────────────────┐  ┌──────────────────┐ ┌──────────────────┐
│ (1) Trees or shrubs│ (2) Vines or herbs│  │ (1) Trees or shrubs│ (2) Vines or herbs│
└──────────────────┘ └──────────────────┘  └──────────────────┘ └──────────────────┘

┌──────────┐┌──────────┐ ┌──────────┐┌──────────┐ ┌──────────┐┌──────────┐ ┌──────────┐┌──────────┐
│(1) Trees ││(2) Shrubs│ │(1) Vines ││(2) Herbs │ │(1) Trees ││(2) Shrubs│ │(1) Vines ││(2) Herbs │
└──────────┘└──────────┘ └──────────┘└──────────┘ └──────────┘└──────────┘ └──────────┘└──────────┘
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PLANT SCORING CHART

Name of Plant	Numerical Values of Choices Made									Total Score

EXPERIMENTS

WITH A SLIME MOLD

Living material assumes many shapes and sizes as well as many degrees of complexity. We can study living material in one of its simplest forms in a group of plants called the slime molds. These plants are found in cool, damp, shaded areas of the woods, growing on rotting logs and other decaying vegetation. During certain stages the organism may appear to you to be more animal than plant because it is able to move about, much like an amoeba. The amoeboid stage is called a **plasmodium.** It lacks cell walls, and many small nuclei are scattered through the cytoplasm of the plasmodium. At another stage in its growth the plasmodium produces fruiting bodies which are very plantlike because they form thickwalled spores.

■ The purpose of this exercise is to study the reactions of a slime mold plasmodium to various stimuli and to observe the plantlike and animallike characteristics of a slime mold.

MATERIALS

A culture of the slime mold, *Physarum polycephalum,* on filter paper

One Petri dish containing non-nutrient agar medium for each team of students

Acetic acid

Compound and stereoscopic dissecting microscopes

Teasing needle

Source of heat (Bunsen burner or alcohol lamp, candle, etc.)

Glass rod

Oatmeal

Fruiting bodies of some slime molds

Razor blade

Slide and cover glass

Detergent solution

PROCEDURE

You will receive a Petri dish containing a thin layer of non-nutrient agar medium. You will also be given a small square of filter paper which has the dried plasmodium on it. Place the square of paper containing the plasmodium in the center of the plate. Dampen the paper with a few drops of water. Replace the top of the Petri dish and allow the culture to remain in a cool, moderately well-lighted place for 2 or 3 days.

Each day, examine the plate for signs of growth. Make sketches in your notebook of any growth you are able to observe. What form does this growth take? (1)

Within several days you should be able to find large masses (1 cm to 2 cm) of new plasmodia which look like jellylike growths. Observe the activity of the plasmodium with the low-power objective of the compound microscope. Observe the movement of the cytoplasm. Write in your notebook a paragraph describing the movement you see in the slime mold plasmodium. (2)

Puncture a large branch of the plasmodium with a needle. Is the hole repaired? (3)

Using a razor blade, carefully cut off several small pieces of the plasmodium and leave them close to the main body. Do the pieces rejoin the main body? (4)

Is the main body of the plasmodium an individual? (5) What about the separate pieces? (6)

Warm one side of the plate. Does the organism react to the heat? (7) You may wish to observe the reaction to cooling as well as to warming.

Dip a glass rod in acetic acid and touch the plasmodium. Is there a reaction? (8)

Place a small amount of oatmeal on one side of the plasmodium. Observe the plant for 5 to 10 minutes. Is there a reaction? (9)

Now look at the fruiting bodies of a slime mold under a dissecting microscope. These

tiny bodies are found on rotting wood and leaves in woods, ravines, etc. They are very common in nature but so small that they are easily overlooked. The fruiting bodies are produced by the plasmodium. Put a fruiting body on a microscope slide, add a drop of detergent solution, and cover with a cover glass. Using the high power of your microscope, find the small rough-coated spheres. These are spores —asexual reproductive structures produced by many plants. Under proper conditions the spores of the slime mold will give rise, directly or indirectly, to a new plasmodium.

If you had seen only the plasmodium you might have classified a slime mold as an animal. On the other hand, if you had seen only fruiting structures you might have guessed it was a plant. Knowing that it has both plant and animal characteristics, how would you classify a slime mold? (10)

GROWING MUSHROOMS

Of all of the living things that grow, fruiting bodies of mushrooms are among those that grow the fastest. They seem to appear as if by magic in a matter of hours and then to disappear almost as rapidly. By using specially prepared mushroom trays you can study the growth of mushrooms and the structure of their fruiting bodies.

■ The purpose of this exercise is to measure and graph the growth rates of a fruiting body and to study its structure.

MATERIALS

Special mushroom kit
Squares of black paper 8 cm x 8 cm, one per student
Razor blade
Tumbler or similar cover for mushroom cap
Centimeter ruler

PROCEDURE

It will take approximately 30 days after you start watering the compost in the mushroom kit before the first fruiting bodies start to appear. Sooner or later there will be enough fruiting bodies so that each student can participate in the experiment.

Because there will be a large number of fruiting bodies formed, they can be picked at various stages for observation. Using a razor blade, cut the younger fruiting bodies lengthwise to see the internal structures. The fruiting body is made up of a stalk and an umbrella-like cap. Look on the underside of the cap and find the gills. In the button stage the gills are covered by a membrane. What happens to the membrane as the fruiting body gets older? (1) What is the function of the covering membrane? (2)

Make a graph on which to record the rate of growth of a mushroom fruiting body. Measurements should start as soon as the button stage of the fruiting body appears at the surface of the compost. Two measurements should be made, one for the height of the fruiting body and one for the diameter of the mushroom cap. The second measurement cannot be started until the top of the mushroom fruiting body is approximately 1 cm above the level of the compost.

When the fruiting bodies have reached their maximum height and the cap is fully expanded, cut the cap from the stalk and put the cap, gill side down, on a piece of black paper. Cover the cap with a tumbler or some other object that will protect the cap from air currents. Set the cap, paper, and tumbler on a shelf and observe it the next day for the "print" you have made of the cap. What structures formed by the cap made the "print" on the black paper? (3) Where was this powder formed by the cap? (4) What is the function of a mushroom fruiting body? (5) What structures present in the compost gave rise to the fruiting structure? (6)

When the growth in height and growth in diameter of the cap of the mushroom fruiting body have been plotted on the graph, you should be able to see the relationship between growth in height and expansion of the cap. What is the relationship? (7) How does it affect the dispersal of the reproductive products formed on the gills? (8)

GREEN ALGAE—

SIMPLE AND COMPLEX

Although there is great variability in structure and reproduction among the green algae, they are alike in the biochemistry of their photosynthetic pigments and in their stored food, which is true starch. Because of these similarities it is believed that the green algae represent the type of organisms that were ancestors of our green land plants.

■ The purpose of this exercise is to give you a basis for comparison between the green algae and the evolutionarily more advanced green land plants. It is also designed to introduce you to the structure and reproduction of these abundant organisms.

MATERIALS

Pure culture of living *Chlamydomonas* in water

Pure culture of living *Oedogonium* in water

Living or preserved plants of *Ulva*

Demonstration slides showing the sexual stage of *Oedogonium*

Slide and cover glass

Compound microscope

Pipette

Dissecting needle

Chlamydomonas. Using a pipette, take a drop of *Chlamydomonas* culture, place it on a slide, and cover it with a cover glass. Observe first with low power to find motile cells. Then switch to high power and look at the detailed structure of this alga.

What characteristics of *Chlamydomonas* are like those of animals? (1) Which are like those of plants? (2) Make a drawing of a *Chlamydomonas* cell, showing only those structures you can see through the microscope! What happens

to the *Chlamydomonas* on your slide if you allow the water to evaporate? (3)

Ulva. This is a marine green alga found commonly in the tide pools of both Atlantic and Pacific shores. In the tide pools *Ulva* grows attached to rocks by a special structure called a holdfast.

Take a small fragment of *Ulva* and mount it in a drop of water on a slide. Cover with a cover glass. Examine the fragment of *Ulva* with low and then high power of your microscope. Describe the organization of the *Ulva* plant body (thallus). (4) How does it compare with the organization of *Chlamydomonas?* (5)

Oedogonium. *Oedogonium* is a common green alga growing in fresh-water ponds, lakes, and lagoons. Because it is common and you can get fresh material, we have chosen this one for you to study. Compare its structure with that of *Chlamydomonas* and *Ulva.* We want you to decide which of these algae is more complex in structure—which is more highly evolved.

Using the tip of a dissecting needle take a *few* filaments of *Oedogonium* and place them in a drop of water on a clean glass slide. Observe under low, then under high power of the microscope.

The most conspicuous part of the cell is the green-colored body—the chloroplast. Describe the shape of the chloroplast. (6) What is the arrangement of cells in this alga? (7) What would happen to this filament if you took it out of the water and put it on a dry surface? (8)

Run a little iodine solution under the cover glass, using the technique described in Exercise 3-4 (Figure 3-4-2). Where in the cell is the starch located? (9)

If the fresh material of *Oedogonium* lacks reproductive stages, look at the demonstration slides set up by your teacher. Find an enlarged cell whose cytoplasm contains a large amount of stored food. This is the female reproductive

cell. What would this cell be, prior to fertilization? (10) After fertilization? (11) Look for a pore on the side of the reproductive cell. What do you suppose the function of the pore might be? (12) Find the cells that produce the sperms. Are the cells producing sperms larger or smaller than the female egg cells? (13)

The seashore is an excellent place to find a great variety of algal types. In addition to the green alga *Ulva*, you may find marine algae that are brown or red. Some of these are very complex in structure. They may have parts that resemble stems and leaves of highly evolved plants. You can get a better idea of how complex they are by using the score chart in Exercise 12-1. Compare their scores with those of other algae like *Oedogonium* or the score for the flowering plant.

ALTERNATION

OF GENERATIONS

Mosses and liverworts are of interest not only because they are primitive land plants but also because they have life cycles with a distinctive alternation of generations. This is a condition peculiar to many plants in which there is a multicellular phase (sporophyte) that produces spores, alternating with a multicellular phase (gametophyte) that produces gametes (see Chapter 13 of the textbook). We will pay special attention to the alternation of generations in bryophytes, ferns, and seed plants.

■ The purpose of this exercise is to demonstrate the alternation of generations in a green plant and to correlate this phenomenon with reproduction.

```
MATERIALS

Moss plants with sporophytes
Moss plants with male sex organs
Moss plants with female sex organs
Four fresh moss plants
Dissecting needle
Slides and cover glasses
Compound microscope
Demonstration slide of filamentous stage
    of moss (whole mount)
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PROCEDURE

We are used to seeing moss plants growing in clumps and forming mats of plants on logs and on the forest floor. These plants, like the liverworts, grow best in damp, shaded environments. A clump of moss plants is composed of many, many, gametophytes all growing close together. Sporophytes grow out of the tops of the gametophytes, and they are pro-

duced in large enough numbers to look like hairs growing out of the clump of moss plants.

Sporophyte. Take a moss plant with a sporophyte attached. Pull the filamentous stalk of the sporophyte out of the leafy shoot of the gametophyte. The two generations are now separated.

The sporophyte part of the moss has a smooth, leafless stem terminated by a little capsule. With a dissecting needle, break open the capsule of the sporophyte into a drop of water on a slide, and apply a cover glass. What do you think the powdery material in the capsule should be if this *is* a sporophyte? (1) With the high power of the microscope look at the spores. These tiny cells will produce the new gametophytes. How are they distributed in nature? (2) Do they have thick or thin walls? (3) How are they adapted for life on land? (4)

Gametophyte. The spores of most bryophytes germinate readily on damp soil. Here they produce a filamentous stage that looks more like a branching green alga than a part of a moss plant.

The filamentous stage gives rise to the leafy shoot of the gametophyte. This is a photosynthetic, independent structure. How does this leafy shoot obtain water for growth? (5) It is this part of the plant which produces reproductive *organs* and gametes—thus it is called the gametophyte. Let us take an ant's-eye view of the sex organs, which are produced at the upper end of the leafy shoot.

The best way to study these reproductive organs is to dissect or squeeze them from the tips of the leafy shoot. Take a male or female plant so that the tip is between your thumb and forefinger (Figure 14-1-1). Squeeze the tip and at the same time roll it between your thumb and forefinger. Now submerge the tip of the leafy shoot in a drop of water on a microscope slide and tease out the fragments from
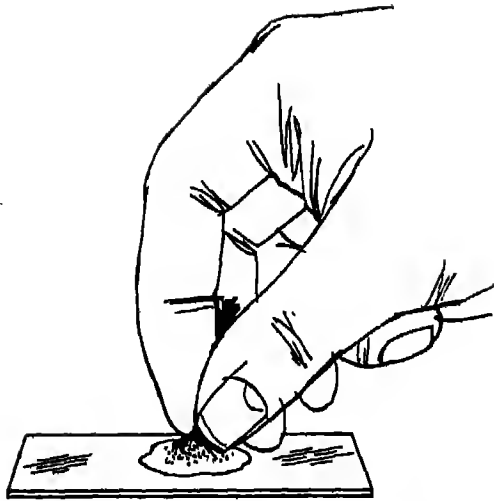
Figure 14-1-1 Squeezing a moss.

the tip of the shoot with a needle. Some of the fragments will be the moss sex organs. Follow the same procedure for both male and female moss plants. Put a cover glass over the fragments from each plant and observe the preparations under the low power of your microscope.

The male sex organs are simple, saclike structures. In nature they produce large numbers of sperm cells. The female sex organs are flask-shaped and have long, twisted necks. An egg is formed within the swollen base of the female organ.

How does a sperm reach the egg for fertilization? (6) Would you expect to find moss plants growing where there was little or no water present? Explain. (7) The union of egg and sperm results in a cell called the zygote. Where is the zygote formed? (8) Into what does the zygote grow? (9) Which generation is initiated by the zygote? (10) In alternation of generations in a moss, which is the predominant, independent generation? (11) Which is the less conspicuous one? (12) How does the life cycle of a moss (with alternation of generations) compare with your own life cycle (with no alternation of generations)? (13)

A PRIMITIVE VASCULAR PLANT

Vascular plants have inhabited the earth for at least 400 million years. Some think the evidence is strong that they have been around even longer. Of the 350,000 different kinds of plants, two thirds of them are vascular plants. It would seem that those land plants with vascular tissue were more successful than those without them. Can you think of a reason why this should be so? (1)

Ferns, like mosses, are of no great direct economic importance. Ferns help form soil and hold it in position, and in addition, ferns are grown extensively as ornamental plants. In the distant past, during the Coal Age which reached its peak 280 million years ago, ferns and many other primitive vascular plants contributed vegetable material to the formation of coal.

It is a mistake, however, to assume that the importance of a plant or group of plants can be established on the basis of economic value alone. Ferns excel in beauty of foliage, and they are among the patriarchs of land plants, having arisen in the Devonian period, approximately 350 million years ago. They are not the most primitive vascular plants, but they are similar in structure and reproduction to some of the most ancient vascular plants.

■. The purpose of this exercise is to become familiar with a primitive vascular plant and its method of reproduction.

MATERIALS

Whole fern plants

Fertile fern fronds

Living fern gametophytes

Demonstration slide of cross section of fern stem

Demonstration slides of fern gametophytes

Slide and cover glass

Compound microscope

Stereoscopic dissecting microscope

PROCEDURE

The Sporophyte. Examine an entire fern plant which has been removed carefully from the soil. Notice the horizontal underground stem, the roots, and the leaves. Where are the youngest leaves? (2) What shape are they before they are fully developed? (3) In the alternation of generations the fern plant is the mature sporophyte. How does it compare with the sporophyte of a moss plant in complexity, longevity, and method of nutrition? (4)

Now observe the upper and lower surfaces of the leaves. Notice that some of the leaves have small brown dotlike or elongated structures on them which are sometimes mistaken for parasitic insects or fungi. Scrape some of the brown material onto a slide and prepare it for examination under the microscope. The small, stalked structures seen are spore cases (Figure 14-2-1). Notice the row of thick-walled cells across the top and back of the spore case. Some of the spore cases probably will have been broken by handling and the spores scattered on the slide. Other spores can be observed still in the cases. What part of the moss sporophyte is similar in function to the spore cases of ferns? (5)

Your teacher will have some demonstrations showing a cross section of fern stems. Find the vascular tissue (the conducting tissue) which may be in the form of a "C" or a ring of bundles within the stem. Some cells of the vascular tissue are empty, thick-walled cells. These are the cells of xylem which provide a pathway for the movement of water and solutes from the roots to the leaves.

Associated with the xylem is the **phloem**. The phloem consists of thin-walled cells forming a layer around the xylem. Phloem is the part of vascular tissue that conducts soluble foods manufactured in the leaves to other parts of the plant.

The Gametophyte. Spores which settle from the air in moist, shaded places germinate to form green, generally heart-shaped plants about as large as a fingernail. Each of these

Leaf blade
Lip cell
Open spore case
Spores
Cheek cell
Annulus
Stalk
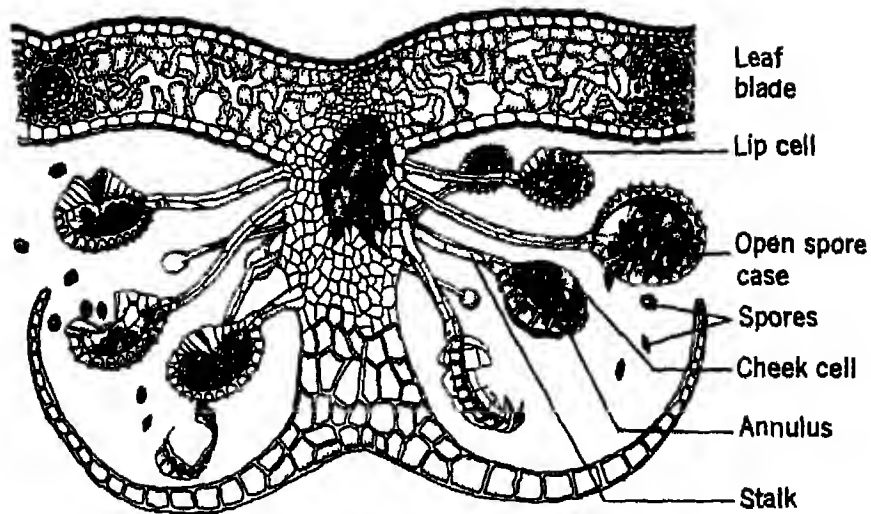
Figure 14-2-1   Cross section of a fern leaf showing spore cases.

structures is a mature **gametophyte.** Would it be easy to find where a fern gametophyte grows in the woods? Explain. (6) How does the gametophyte of a fern compare with that of a moss in food-getting, longevity, and function? (7)

If living gametophytes are available, put one of them on a glass slide and examine it first with the dissecting microscope. Look at the lower surface. What structures are present for water absorption? (8) In what other plants have you seen these structures? (9) In addition to the absorbing structures, sex organs are also located on the lower surface. To see these, use the low power of your compound microscope.

The male sex organs are dome-shaped structures which generally develop in advance of the female sex organs. Look for the male sex organs near the margins of the gametophyte. Crush a male sex organ in a drop of water on a clean glass slide. Look for sperm cells in the

water. They are quite small. Is there any movement of the sperm cells? (10) What is your conclusion about how sperms reach the egg cells in a fern plant? (11) How does this compare with the situation in an alga, and in a moss? (12)

The female sex organs are flask-shaped structures with the enlarged basal portion buried in the tissues of the gametophyte and with the neck projecting above the surface. The enlarged basal portion contains an egg. If you cannot find the female sex organ on the living plant, look at the demonstration slide. The egg is fertilized inside the female sex organ. What name is given to a fertilized egg? (13) What generation, in the alternation of generations, is initiated by the fertilized egg? (14) Which generation is the predominant one in a fern life cycle? (15) Which generation is photosynthetic? (16) Why do ferns normally not inhabit dry environments even though their sporophytes have vascular tissue? (17)

THE IMPORTANCE OF SEEDS

Seed producers are the most highly developed group of plants. Spores of mosses and ferns are left to shift for themselves as soon as they become separated from parent plants. Parent plants provide these offspring with very little food reserve or protection against a variety of environmental dangers. As a result, most of the young die before reaching maturity, and if it were not for the millions of spores, eggs, and sperms produced and the survival of a few by chance, the species involved would perish.

In seed plants better provision is made for the protection, nutrition, and resulting survival of individual plants than in the algae, mosses, and ferns. Fertilized eggs and embryos are not exposed directly to predators and other environmental hazards. Instead, the embryo of a seed plant is surrounded by protective structures, among them the seed coat, and sometimes an additional food supply (endosperm), both provided by the parent plant. Fewer offspring are produced than in lower plants, but a greater proportion survive. The embryo, endosperm, and seed coat make up the seed.

In the majority of higher plants, seeds are given additional protection by surrounding tissues of a vase-shaped, central portion of the flower (the ovary), which at maturity is usually called the fruit.

In approximately 600 species of seed plants an ovary is lacking. Instead, these plants produce their seeds on cone scales. Because the seeds of these plants are not covered by an ovary wall, they are called gymnosperms from the Greek words *gymnos* (naked) and *sperma* (seed). All other seed plants are angiosperms, from *angios* (vase), referring to the vase-shaped ovary at the base of the pistil.

The pine has been chosen as a representative gymnosperm because of its common occurrence, its economic importance, and because it illustrates very well the type of reproductive cycle and vegetative structures found in the conifer group.

■ The purpose of this exercise is to observe the structure of an advanced vascular plant and to study its reproductive cycle for comparative purposes.

MATERIALS

Pine branch with cones
Pollen cones
Young seed cones
Old seed cones
Pine seeds
Stereoscopic dissecting microscope
Compound microscope
Slide and cover glass
Razor blade

PROCEDURE

The Pine Branch. Generally the end branches are elongated, but the side branches remain short and spurlike. Notice the brownish, inconspicuous leaves at the base of needle-bearing branches.

Needle leaves in different species may vary from a single leaf on each short basal stalk to as many as eight. Two, three, and five needles are the most common. How many leaves are borne on each stalk in the species of pine that you are observing? (1)

Most broad-leaved trees of temperate regions shed their leaves each year, but needle leaves may remain on the pine for periods ranging from 2 to 14 years. They are shed gradually, although some species may show a more marked seasonal shedding of branches and leaves than others. Notice old scars on the stem where leaf-bearing branches were once attached. How would you describe the arrangement of these scars: opposite, alternate, or spiral? (2) Identify the part of the stem which was produced during the last year of growth. In a similar manner attempt to identify the part of the branch which is two years old. Can

you estimate the age of the oldest needles on the branch in your possession? (3)

The pine tree with its roots, trunk, and branches is the mature sporophyte. How does this compare with the sporophyte of a fern as to size, and method of nutrition? (4)

Pine Cones. Seed cones are produced singly or in groups of two to five. Pollen cones, on the other hand, usually occur in closely packed clusters of ten or more near the tip of a branch. Can you give any explanation of why it might be advantageous to the pine to produce more of one kind of cone than the other? (5)

The small, spirally arranged cone scales making up most of the pollen cone are modified leaves so specialized that they hardly resemble leaves. Place one of these leaves under the dissecting microscope. Notice the two elongated spore cases on the lower side of each cone scale. Crush a spore case on a clean glass slide, use a cover glass, and examine the specimen under the high power of the microscope. What are the small structures with little bladders? (6) What part do they play in the life cycle? (7)

Now let us look at a young seed cone. The spirally arranged cone scales are fleshy and may prove to be a little difficult to pry apart, but if we use care we can do it and locate the ovules. At the base of each cone scale on the upper side you should find a pair of white or cream-colored bumps. These are the ovules that will grow into seeds. How are the ovules of a pine protected? (8) How do you suppose the sperm cell reached the egg in the ovule? (9) How are the seeds, resulting from growth of ovules, shed from cones? (10) To answer this last question look at an old seed cone which has shed most of its seeds.

The Seed. Here is the start of a new pine tree. Let us find out if it has a good start—if it can survive the rigors of the environment before growing into a new tree. Break the seed coat and remove it. Inside you will find a white, fleshy tissue. This is like a lunch basket in the seed. It is stored food or endosperm. How is the endosperm used? (11) The answer to this question lies in the very center of the food itself. With a sharp razor blade make a lengthwise cut through the center of the endosperm and look at the cut face. If you have made the cut properly you will see the embryo, which is surrounded by the stored food. Now you can answer Question 11. Which would you think has the better chance of survival, the spore of a fern or moss, or the seed of a pine? Give your reasons. (12)

Alternation of Generations. If the pine tree with its conspicuous parts is the sporophyte, then what has happened to the gametophyte generation? We are not going to try to answer this question here because to do so would require a more detailed microscopic investigation of the reproductive parts. You might guess from the statement that a microscope is needed to see them, that the gametophytes are very small and are included somewhere in the cones or their products.

THE PIGMENTS IN A LEAF

Thus far, our discussion of photosynthesis has been rather abstract. Let us take some green leaves, extract the pigments, and see how the pigments function in photosynthesis. The first thing we have to do is to find leaves from which we can easily remove the pigments. If it is winter and there is no green foliage available, a box of frozen spinach will serve. Otherwise, bean leaves, leaves of geraniums, or some Virginia creeper from the walls of your school will provide suitable material.

■ The purpose of this exercise is to determine what pigments occur in green leaves and to enable you to master the techniques of pigment extraction.

MATERIALS

Eight living green leaves
Hot plate or water bath
Two 600-ml beakers
Capped medicine bottle, 250-ml (8-oz)
Flask, 250-ml
Graduated cylinder, 250-ml
Medicine-dropper pipette
130 ml carbon tetrachloride (CCl_4)
200 ml of 95% ethyl alcohol
Large forceps or tongs
Pot holder
Cotton

PROCEDURE

Turn on the hot plate. While it is warming up, half fill one of the 600-ml beakers with water and start it heating. Take the other 600-ml beaker and measure 200 ml of 95% ethyl alcohol into it and put it on the heat. CAUTION: *Be careful of the alcohol—use a hot plate, or heat it in a water bath. Do not heat it over an open flame.*

Take about eight leaves (or leaflets if you use Virginia creeper), crumple them up in

your hands, and put them in the hot water. Do not leave them in the hot water too long. A minute or two will be sufficient. Does any pigment come out into the water? (1) What can you conclude about the solubility in water of the green pigments? (2)

With the large forceps or tongs transfer the wilted leaves from the boiling water into the hot alcohol. Boil the leaves for several minutes and, at the same time, stir them with the tongs. Now what can be said about the solubility of leaf pigments? (3) What color are the leaves after about 5 minutes of boiling? (4)

At the end of 5 minutes you should have extracted most of the pigments. Allow the alcohol to cool a little. Using a pot holder, pick up the beaker of hot extract, measure 100 ml of extract, and put it in the 250-ml (8-oz) medicine bottle. Put the balance of the extract in the flask, stopper it with a cotton plug, and store it in a refrigerator for future use.

Different substances show different degrees of solubility in the same solvent. For example, we would not think of trying to remove a grease stain from our clothes with water; we would use carbon tetrachloride instead. Because of the differences in solubility of different substances (**differential solubility**), it is possible to separate substances which are mixed together in the same solution.

When you look at the pigment extract obtained from the leaves, its color would seem to indicate that it is composed of green chlorophyll. But is it really all chlorophyll? Let us use the principle of differential solubility to find out.

To the 100 ml of pigment extract in the 250-ml medicine bottle add 130 ml of carbon tetrachloride. Take care not to inhale the fumes or to get any of it on your skin. Put the top back on the bottle and shake the contents. If there is no sign of a separation into two layers take two or three medicine droppers full of water and add this to the solution. When you shake the bottle of extract it should have a cloudy appearance. If it turns clear and there is still

no sign of a separation, add a little more water. If the cloudy appearance remains after shaking the solution, put the bottle down. You are now ready to make some observations.

This time the cloudiness will disappear rather slowly. When the separation is complete you will observe two layers in the bottle, one alcohol, the other carbon tetrachloride. Describe the colors of the layers. (5) What are your conclusions now about the number of pigments present in the extract? (6) Which pigments are more soluble in carbon tetrachloride? (7) Which ones are more soluble in alcohol? (8)

CHLOROPHYLL

AND PHOTOSYNTHESIS

You may have noticed that some plants have variegated leaves. These are leaves in which chlorophyll is lacking in some parts and present in others. The silver-leaf geranium and certain kinds of *Coleus* have variegated leaves.

■ The purpose of this exercise is to experiment with leaves of one of these plants to determine whether or not the presence of chlorophyll in the leaf is really important in photosynthesis.

MATERIALS

Plant with green-white variegated leaves
Hot plate
Two 600-ml beakers
95% ethyl alcohol
Tongs or large forceps
Petri dish
Iodine solution
Pot holder

PROCEDURE

Pick a fresh, living, variegated leaf and make an outline drawing showing the distribution of its green pigments. You will want this later for comparison.

Next, we want to remove the pigment from the leaf by using the same procedure followed in Exercise 15-1, where the leaf is wilted in boiling water and the pigments are removed in boiling alcohol. When the leaf is well bleached remove it from the hot alcohol and put it in a Petri dish. Flood the leaf with iodine solution. Wherever starch is present, the leaf tissue will turn brown to purple in color.

Compare the distribution of starch in the leaf with the distribution of the chlorophyll. What is the relationship of the two patterns? (1) Keep in mind that starch is an indirect product of photosynthesis derived from the combining of glucose molecules. Why not test for sugar instead of starch? (2) What can we conclude about the importance of chlorophyll in photosynthesis? (3)

LIGHT IN CARBOHYDRATE SYNTHESIS

In Chapter 15 of the textbook we learn that green leaves absorb certain wavelengths of light and reflect or transmit others. In the presence of the absorbed light, living green cells with a sufficient supply of carbon dioxide and water synthesize carbohydrates. One of the first visible products of carbohydrate production is starch in the form of small grains. You may have already seen these grains in potato or some other plant part. Starch, as a product of photosynthesis, is easy to demonstrate with the iodine test. Its presence or absence in the green leaf will serve as an indication of photosynthetic activity.

■ The purpose of this exercise is to see what effect light has on green plant parts.

MATERIALS

Geranium plants with light screens
Hot plate
Two 600-ml beakers
95% ethyl alcohol
Tongs
Petri dishes
Iodine solution

PROCEDURE

The plants with the light screens attached to their leaves have been prepared ahead of time by your teacher. You must be told how they were treated before you will be able to draw any conclusions at the end of the experiment.

Your teacher has put the geranium plants, minus the light screens, in a dark, light-tight room for 36 hours. What effect will this have on photosynthesis? (1) What will the plant use as a source of food during that period? (2) At the end of the period in the dark, the light screens were put in place and the plants were exposed to light for 12 hours.

Before you start the experiment, examine the light screens. They are made of opaque paper or photographic negatives that exclude light from part of the leaf.

Pick two leaves from the plant, one with and one without a light screen. Remove the light screen, then put both leaves in boiling water, followed by boiling in alcohol to remove the chlorophyll. Test both leaves for the presence of starch by flooding them with iodine solution. Check your procedure with that outlined in Exercise 15-1.

Compare the two leaves. What indication is there that light is required for the synthesis of food in the leaf? (3)

THE STOMA—

GATEWAY INTO A LEAF

It has been demonstrated that a particular part—a very small part—of our atmosphere plays a very big role in photosynthesis. It is easy for us to think of roots as absorbing organs responsible for obtaining water and minerals for the plant. It is a little less obvious that leaves are also absorbing structures—structures well designed to absorb a gas from the atmosphere.

■ The purpose of this exercise is to investigate the carbon-dioxide-absorbing structures of a leaf.

PROCEDURE

Consult Figure 15-4-1 for the method of tearing a leaf at an angle. Hold the under surface of the leaf toward you. The tearing action

MATERIALS

Compound microscope
Salt solution, 5%
Fresh leaves
Medicine-dropper pipettes
Filter paper

Sharp razor blade
Slide and cover glass
Paper toweling
Plant growing in medium light

A

B

C

D
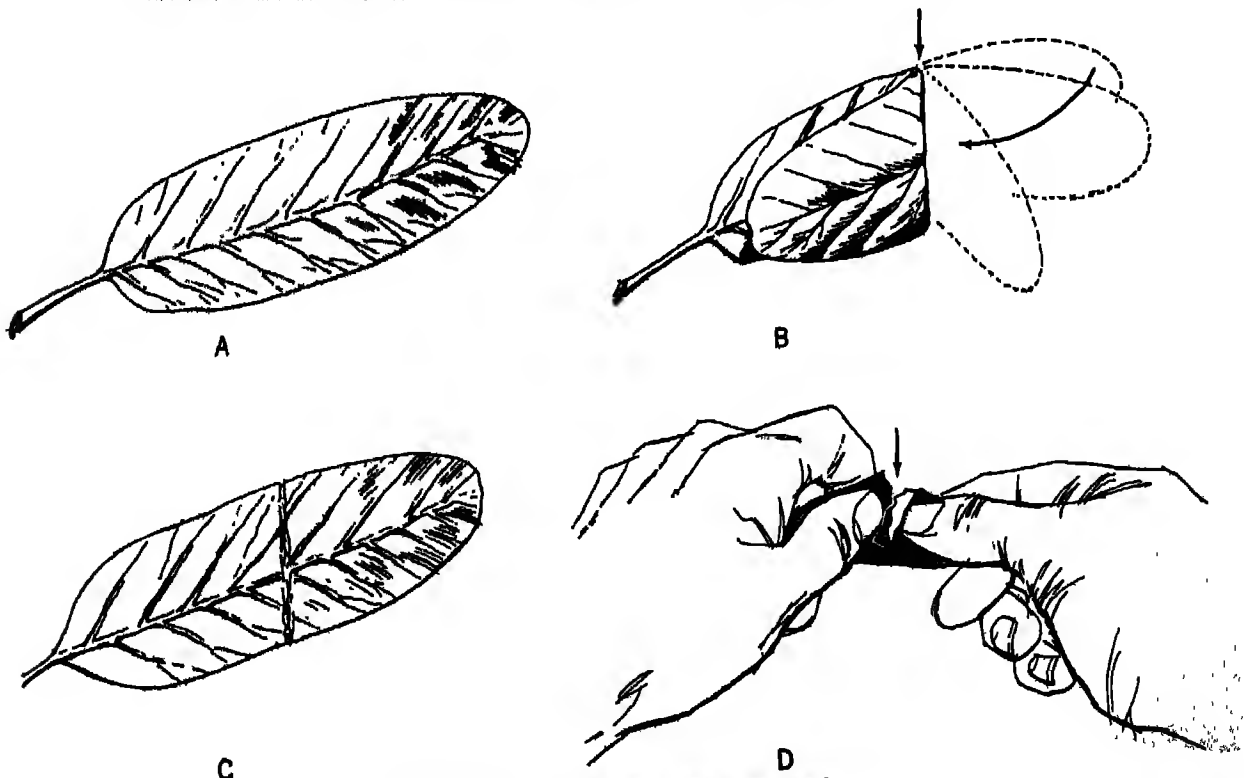
Figure 15-4-1 Tearing a leaf at an angle.

should peel off portions of the lower epidermis which will appear as a narrow colorless border along the torn edge. (Figure 15-4-1.)

Using the sharp razor blade, cut off a very small fragment of the colorless layer and immediately place it in a drop of water on a slide. (Do not allow the fragment to dry.)

The Leaf Epidermis

Look at the specimen under the low power of your microscope. You will see the lower epidermis, consisting of cells which may look like pieces of a jigsaw puzzle. Are there any chloroplasts in these cells? (1) Look for smaller, bean-shaped cells which occur in pairs. These are **guard cells**. Do they contain chloroplasts? (2) To discover the answer to this question you will have to look at the specimen under high power. Focus carefully on a pair of guard cells and compare the thicknesses of their inner and outer walls. Are the walls of a guard cell uniformly thick? (3) You should be able to see an opening or pore between the inner walls of a pair of guard cells. The opening is called a stoma (stomata is the plural).

Make an outline drawing of a pair of guard cells and the surrounding epidermal cells.

Opening and Closing of Stomata

By now you have observed that there are many, many holes, which we call stomata, in the epidermis of a leaf. As long as these holes, or pores, are open, there will be an opportunity for a free exchange of gases of the external atmosphere and the internal atmosphere of the leaf. Stop and think for a moment of the kinds of gases that will or can be exchanged. Make a list of these gases. (4) Which one of them do you recognize as being important in photosynthesis? (5) What percentage of the earth's atmosphere is composed of this gas? (6) If stomata are open to allow this gas to diffuse into the leaf, what gases will diffuse out? (7) Can the plant regulate the exchange of gases? (8) It is possible to find the answer to this question by making the following simple observations of stomata.

Functioning of Stomata

Remove a fragment of lower epidermis from a leaf on a plant growing in medium light. Prepare a wet mount of the fragment and observe it under high power. Are the stomata open? (9)

Using the technique illustrated in Figure 3-4-2 in Exercise 3-4, absorb the water from beneath the cover glass with a piece of paper toweling and replace it with salt solution. Make another outline drawing of a pair of guard cells and compare it with the drawing you have already made. Are the stomata open? (10) Account for any changes you have induced by using salt solution. (11) How might these same changes be brought about by the normal activity of the plant? (12)

THE LEAF—

A PHOTOSYNTHETIC ORGAN

We have already studied the various substances (chlorophyll, carbon dioxide, and water) which are required in photosynthesis. Except for the stomata, we have not concerned ourselves with the structure of a leaf as it is related to this wonderful process.

By now you have worked with the leaves of many kinds of plants and, although there is much variability in their form and structure, we can usually recognize a flat, bladelike structure. This may or may not be attached to a leaf stalk (petiole).

Look at a plant with its attached leaves. Of what advantage is the flat, often thin, blade to the photosynthetic capacity of a plant? (1) What are some of the ways leaves are arranged on the stem? (2) Is this in any way related to the photosynthetic capacity of a plant? (3)

■ The purpose of this exercise is to relate the structure of the leaf to its function in photosynthesis.

MATERIALS

Growing plant with numerous leaves
Prepared cross section of a leaf
Compound microscope

PROCEDURE

Your teacher will give you a prepared cross section of a leaf. Find the section under the high power of your microscope and consider its structure as a light absorber, water supplier, and absorber of carbon dioxide.

The Leaf as a Light Absorber

Remember that chlorophylls, the pigments responsible for light absorption, are located in chloroplasts. Find the chloroplasts in the cells of the leaf. What shape are the chloroplasts? (4) In Figure 15-5-1, draw in chloroplasts in a few of the appropriate cells. What cells contain the chloroplasts in the leaf? (5) Find the vertically elongated cells (palisade cells) just below the upper epidermis. These are the chief photosynthetic cells of the leaf. How can you tell? (6) Explain how their position in the leaf is advantageous in light absorption.(7)

The Leaf as a Water Supplier

In the centrally located tissues of the leaves you can find veins of various sizes cut in various planes. Find a small vein cut directly across (cross section). The vein is surrounded by a sheath of cells which are also photosynthetic. Notice some empty cells with thick walls in the upper part of the sectioned vein. These are xylem cells. What two functions do xylem cells in a vein perform? (8) The cluster of small, thin-walled cells just below the xylem are the phloem cells of the vein. What function does the phloem have? (9)

Now take a close look at the covering layers of the leaf, the upper and lower epidermis. They can be identified as single layers of cells, one at the upper, the other at the lower surface of the leaf. The outer surfaces of these cells are covered by a thin layer of waxy substance called cuticle. This may appear as a thin yellow layer. In what indirect way does the cuticle of the plant play a part in the efficiency of photosynthesis? (10)

The Leaf as an Absorber of Carbon Dioxide

You have already seen the stomata and guard cells in the lower epidermis of the leaf. Find a sectioned stomatal apparatus in the lower epidermis of the sectioned leaf. You can see that a stoma serves as a direct passageway between the external atmosphere and internal

atmosphere of the leaf. The leaf tissue just above the lower epidermis is often called the **spongy tissue**. What feature of the arrangement of the cells in this tissue gives it its name? (11) Is there any relationship between the arrangement of these cells, the stomata, and carbon dioxide absorption? (12) How is the carbon dioxide actually absorbed? (13)
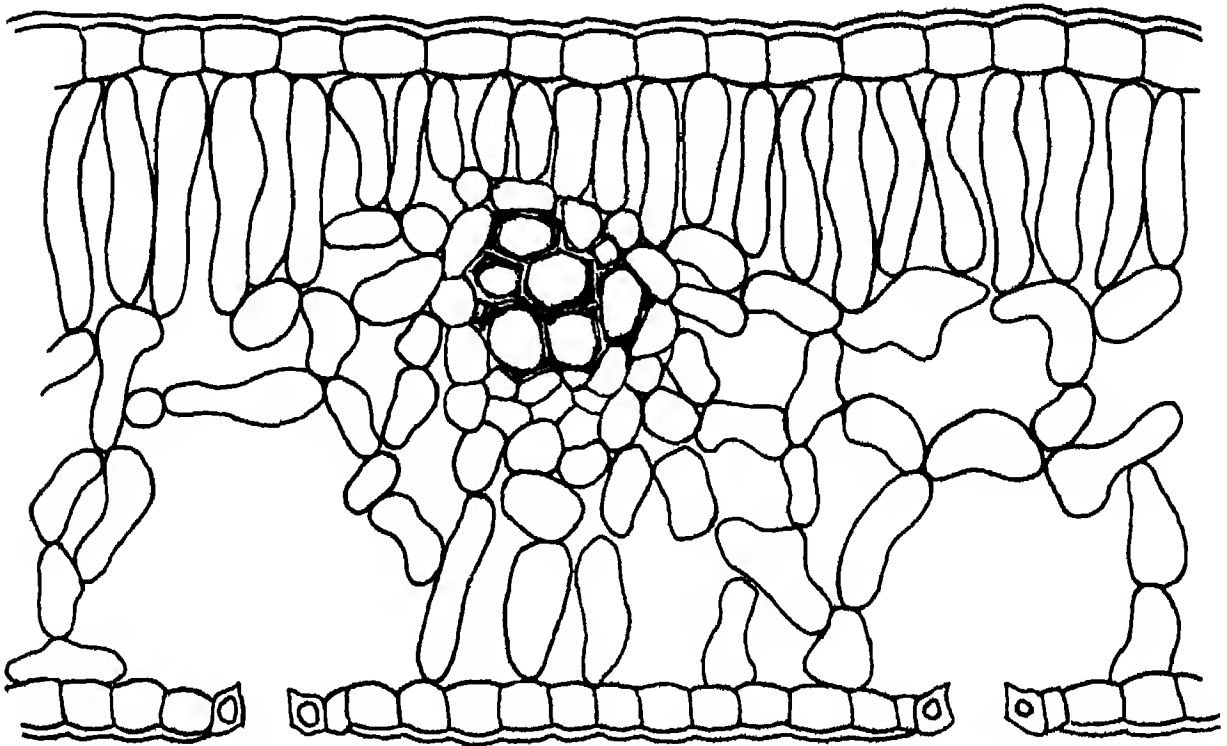
Figure 15-5-1   Cross section of a leaf blade.

THE STEM—

STRUCTURE AND FUNCTION

We might get the idea from previous exercises that the leaf, with its capacity to carry on photosynthesis, is primarily responsible for the nutrition of a plant. To a certain degree this is true. However, what good would a green leaf be without some place to store its food or without structures to furnish it with a source of water to carry on photosynthesis or a stem to hold the leaves up into the light? A glance at a tree or other plant shows us that stems and their branches do support the leaves. The other functions of storage and conduction are less obvious.

■ The purpose of this exercise is to look inside the stem to see how its structure and function are correlated.

MATERIALS

A young bean plant

Prepared cross section of an herbaceous dicotyledonous stem

Colored pencils (red, blue, brown, yellow, green)

Compound microscope

PROCEDURE

We do not have to examine many plants to realize that there are many kinds of stems. There are the sturdy, woody stems of trees and shrubs. Other stems are soft and succulent. Let us look at the stem of a succulent or herbaceous plant and identify the regions of a stem.

As a means of identifying places on a stem, we relate everything to the position on a stem where the leaf is attached. This position is called a **node**. The stem portions between nodes are called **internodes**. Find these regions on a stem. Are all the internodes of the same length? (1) What color is the stem? (2) Judging from its color, what function does it

have in common with a leaf? (3) Do all stems have this same function? (4)

You will be given a prepared slide—a cross section through the internode of a stem—for you to look at under low power. At this magnification you can identify certain general regions and see some tissues that have some very specific functions important in plant nutrition. Before doing this, become familiar with the general regions of the stem so that when we talk about kinds of cells in the epidermis, cortex, vascular bundles, and pith you will know where they are located.

As in the leaf, an herbaceous stem is covered by an epidermis. How many cells thick is this layer? (5) The most conspicuous feature of the cross section is a ring of vascular bundles. Each bundle may be wedge-shaped, with the narrow end of the wedge directed toward the center of the stem. The central region of the stem is composed of large thin-walled cells. This region is the pith. Between the outer edges of the vascular bundles and the covering epidermis you will find a region called the cortex.

Draw a circle at least 13 cm in diameter. Now in the circle draw an outline of the vascular bundles as they appear in your stem slide. Do not draw in any cells.

Supporting Tissues in Stems

Keep in mind that, without support, leaves would not be advantageously exposed to the light. A large tree has many leaves which are supported by a sturdy trunk and branches composed of wood. Wood is composed mostly of supporting cells. Generally speaking, supporting cells, when seen in cross section, have fairly thick walls and look very empty. Using high power, look in the cortex and vascular bundles for cells of this kind. Show in your diagram simply by coloring the regions red, what parts of the cortex and vascular bundle are supporting tissues.

Conduction and Conservation of Water

The leaf requires water for photosynthesis. We know that this water is absorbed by the roots and is conducted through the roots and stem to the leaves. In what structures of the stem would it be logical to look for xylem cells, the cells in which water moves upward in the stem? (6) Are these cells living or dead? (7) Do they have any other function? (8) Have you already colored them? (9) With a blue pencil, color all the parts of your stem drawing representing xylem.

Excessive amounts of soluble carbohydrates are manufactured by the green leaves. Since the leaves of most plants are not constructed to store this extra food, it must be conducted to other storage parts of the plant through a special tissue called the phloem. In what part of the stem is this tissue located? (10) Using a brown pencil, color that part of your diagram which represents the phloem.

Both xylem and phloem conduct quantities of water in addition to food and minerals dissolved in the water. All living cells require water for their various functions, including photosynthesis. Without some kind of waterproofing layer on the plant, the water would soon evaporate from the stem and the plant would wilt and die. Where is the waterproofed layer of the stem? (11) Using a yellow pencil make a line showing its position on your diagram.

Storage of Materials

Excess quantities of carbohydrates manufactured by leaves are usually stored in the form of visible starch grains. Irish potatoes are stems that store abundant starch. In the stem slides, starch grains will probably be stained purple or violet. Using high power, carefully examine cells of the various stem tissues for starch grains. Take a green pencil and color all the storage tissues in the diagram. What region is devoted primarily to storage? (12)

THE ROOT—

AN AID TO PLANT NUTRITION

If you know that plants make their own food by photosynthesis and do not absorb it through their roots, you are doing better than Aristotle or his students, and better than 30% of the students in a freshman class tested recently at a university.

■ The purpose of this exercise is to answer the following questions. Since roots do not absorb food for the plant, what are their functions? Are these functions related to the nutrition of the plant? How are roots designed to accomplish their functions?

MATERIALS

Radish seedlings germinating in a Petri dish
Grass seedlings germinating on water
Prepared slide of a root cross section
Root system of a grass plant
A bunch of carrots
Colored pencils (blue, brown, green)
Forceps
Slide and cover glass
Stereoscopic dissecting microscope
Compound microscope

PROCEDURE

Absorption of Water

The absorption of water and dissolved minerals is one of the important functions of the root. Without water the processes of the plant, including photosynthesis, would soon stop. When we use the word *absorption*, a sponge, blotter, or dish towel may come to mind. Each of these objects is composed of many small particles of skeleton, cellulose, or fibers, which offer a great deal of surface which can absorb the liquid. The more surface, the greater possibility for absorption. We can see right away that the fibrous root system of grass is composed of many small slender roots with a great deal of surface for absorption. How does this compare with the root of a carrot? (1) The carrot seems to have a relatively small amount of surface for absorption. This is due to the fact that you are unable to see some of the smaller branch or secondary roots and root hairs which increase the surface for absorption hundreds of times. Let us look at some roots that have root hairs.

First is a demonstration of radish seedlings germinated on absorbent paper in a Petri dish. (CAUTION: *Do not remove the covers from the dishes.* The drying effect of the air will ruin the demonstration.)

Use the dissecting microscope to look at the cottony mass of root hairs through the cover of the dish. On what part of the root are the root hairs longest? (2) What would happen to the hairs if the plant had been growing in the soil and we pulled it up? (3) You can see that root hairs are delicate structures but it is difficult to tell whether they are multicellular or unicellular.

With forceps pick up a young grass seedling from the water in the dish where it is growing. Mount the seedling in a drop of water on a clean glass slide. *Do not put a cover glass on the specimen at this time.* With the low power of your compound microscope, look for the root tip. Do not confuse the young green shoot with the root tip. When you find the root tip, look along the root until you find some root hairs. Are they multicellular or unicellular? (4) What relationship do they have to the epidermis of the young root? (5) To answer this last question you may have to put a cover glass on the specimen and examine it under high power. Are root-hair cells living or dead? (6) How does the function of the root epidermis with its root hairs compare with the function of the epidermis of stem and leaf? (7)

What process is involved in the absorption of water by root-hair cells? (8) You have studied this process in Exercise 6-1 and Exercise 6-2. Review the conditions which are required for the movement of water into living cells.

Conduction in the Root

Your teacher will provide you with a cross section through the mature region of a root. Start your observations with the low power of the microscope. Find the epidermis, cortex, and pith. Find the xylem in the root. How does its position compare with its location in the stem? (9) Draw a circle about 13 cm in diameter to represent the cross section of the root. Using the blue pencil, color in the location of the xylem on your diagram.

Now look for the phloem cells. What is the distribution of the phloem relative to that of the xylem? (10) What is the function of the phloem? (11) Using the brown pencil, color in the location of the phloem on your diagram.

The Root as a Storage Organ

Roots store food in varying degrees. The fibrous root of grass stores less food than the fleshy root of a dandelion.

We eat many kinds of roots, such as carrots, sweet potatoes, radishes, parsnips, turnips, and beets, because they contain much stored food. Remember, the plant uses this stored food at a later time for its own growth. Examine the cells and tissues of the root with the high power of your microscope, looking for starch grains. What region of the root contains the greatest amount of stored food (starch)? (12) Color this region on your diagram with the green pencil.

One of the obvious functions of a root is the anchorage of the plant, a function quite different from the supporting functions of the stem. What are the noticeable differences between the structure of the stem and root which are related to support and anchorage? (13)

TRANSPIRATION

IN PLANTS

Previous exercises have demonstrated that water is absorbed by roots and moves with some rapidity into the stems and leaves. This supply of water carries dissolved minerals from the soil through the plant. It is used in photosynthesis; it maintains the rigidity of living cells—in general, a supply of water is required for all the functions of the plant. But how does the water move through the plant? How does it get to the top-most leaves of a tree 300 ft tall? Is it pushed up? Could it be pulled up? A tremendous force of some kind is required to move water to such a height.

The movement of water upward in a plant is related to the loss of water from the aboveground parts of the plant in the form of water vapor. The loss of water vapor is called transpiration. Most of the water vapor is lost through the stomata in the leaves; however, some is lost through the cuticle of leaves and stem. Of all the large quantities of water absorbed by a plant, only a very small part, often less than 5%, is used by the plant in photosynthesis and other processes involved in nutrition. The rest is lost by transpiration.

■ The purpose of this exercise is to show that water vapor is lost from leaves, to show how much water is lost from stems and leaves, and to show the force of transpirational pull.

Part A:
Water Loss from Leaves

A good way of detecting the presence of water vapor is by using cobalt chloride paper. In the presence of moisture this blue paper turns pink. It is sensitive to relatively small amounts of moisture that may be in the atmosphere. Because of this, it is best to keep the paper in a calcium chloride desiccator.

PROCEDURE

Cut two 3-cm squares of blue cobalt chloride paper. Put one square on top of a leaf and the other on the bottom. Fold a strip of cellophane over the leaf and papers and fasten cellophane, papers, and leaf together with a clip. If the cobalt chloride paper is pink, heat it over a flame for a minute or two, until it turns blue, before putting it on the plant.

Look for color changes in the cobalt chloride paper. What does the pink color indicate is happening? (1) Why does the paper on the lower surface of the leaf turn pink first? (2) To answer this question think back to the structure of a leaf. Recall that stomata may be on the lower surface of the leaf. Grasses have them on both surfaces and water lilies just on the upper surface.

MATERIALS

Part A
Cobalt chloride paper
Cellophane
Paper clips
Geranium plant

Part B
Balance weighing to the nearest gram

One potted geranium plant
One pot filled with soil
Two plastic bags
Graduated cylinder (250-ml)

Part C
Potometer with a living branch
Potometer modified for use by student
Electric fan

Part B:
Measuring the Volume
of Water Lost

A simple, graphic demonstration can be constructed to show how much water a geranium plant will lose.

PROCEDURE

Water both the potted geranium plant and the pot filled with soil; then enclose each pot with a plastic bag. Tie the plastic bag snugly around the lower portion of the stem of the potted plant, the top of which will protrude from the bag, so that no water can evaporate from the pot. Be sure the pot with soil is also tightly sealed in the plastic bag.

Now weigh the pot containing the plant and also weigh the other pot containing soil only. Weigh to the nearest gram. Record the weights of each one in your notebook. Each day for the next 5 days record the weight of each. If the plant needs watering, measure the amount of water you add and add a similar amount to the pot of soil. As soon as the watering is done, weigh the two pots again and record. Which loses the greater amount of water in the 5-day period—the pot with or without the plant? (3) How can you account for the differences in water loss between the two pots? (4) List all of the processes in the plant that are involved in the water loss. (5)

Explain how transpiration regulates the movement of water in a plant. (6) What factors of the environment regulate the rate of transpiration? (7) When would a plant lose the greater amount of water: on a dry, bright day or a dull, humid day? (8) Be sure you can explain.

Part C:
The Force of Transpirational Pull

This demonstration will be set up by your teacher prior to class. It consists of two parts: one part illustrates the force generated by the plant in moving a column of a liquid; and the second, an apparatus that you can use in trying to duplicate the force exerted by the plant.

Look at the potometer with the living branch and see how it is constructed. You can see that the branch has its cut end inserted through a rubber cork into a bottle filled with water. The

water-filled bottle in turn is connected by a long capillary tube (a tube with a very small bore or hole through it) to a finger bowl also filled with water. The capillary tube substitutes for what part or parts of the plant? (9) Is there a water column in the capillary tube or is it empty? (10) Take a close look, because what you observe is important to an understanding of the factors involved in the upward movement of liquids. What part of the plant's natural environment is represented by the water in the finger bowl? (11)

Look in the finger bowl. Submerged in the water you can see a watch glass filled with mercury. Carefully help your teacher to raise the end of the capillary tube in the water and lower it into the submerged dish of mercury. (CAUTION: *You must be very careful not to let the end of the capillary tube come above the water level in the finger bowl.* If you do, an air bubble will enter the system, interrupting the continuity of the liquid. This will spoil the experiment.) Can you explain why? (12)

Now your teacher will let the electric fan blow on the living branch. What happens to the mercury column when the fan is on? (13) When it is off? (14) Before the end of the class period your teacher will measure, with a meter stick, the maximum height the mercury has ascended in the tube. Mercury is 13.6 times as heavy as water. Knowing this, how high would the plant have moved a water column during the class period? (15) Was the mercury column pushed or pulled up the capillary tube? (16) What process going on in the living branch caused the water and mercury to move in the capillary tube? (17) What do we mean by cohesive force? (18) Explain why the column of water or mercury would not move without this force. (19) What is adhesion? (20) How is adhesion important to this experiment? (21)

While you are waiting for the mercury column to ascend in the potometer with the living branch, let us see how easy it is to pull a column of mercury into a capillary tube of the same size used by the plants. Take a sterilized glass mouthpiece and insert it into the end of the rubber tube. Suck on it as hard as you can. Record the maximum height to which you can get the mercury column to ascend. Multiply this by 13.6. How many meters high could you pull a column of water? (22) Compare this with the plant's performance. Now can you explain how water gets to the uppermost leaves of a 300-ft tree? (23) Does it take a great deal of force? (24)

THE FLOWER

We have all heard the old cliché, "We are learning about the bees and the flowers." It is an interesting fact that the basic principles of sexual reproduction in a flower, a bee, and a human being are indeed the same. Just as in asexual reproduction, sexual reproduction is directed toward increasing the numbers of individuals of a certain kind of flower, bee, or human.

■ Our purpose here will be to examine the structure of a flower and to determine the reproductive functions of its various parts.

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MATERIALS

Sweet pea flower
Some other simple flowers for comparison
Razor blade
Stereoscopic dissecting microscope
Fresh bean or pea pods

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PROCEDURE

Before we dissect the flower to see the parts on the inside, let us look at the outside.

The outermost whorl of floral parts may be green, leaflike parts which protected the flower bud when it was young. These are called sepals. In some flowers, lilies for example, sepals seem to be lacking. Actually they are present and look like an outer whorl of petals. Petals are usually large and colored, and lie just inside the sepals. Both sepals and petals are attached to the enlarged end of a branch. The enlargement is called the receptacle. These three floral parts are called accessory parts because they are not directly involved in sexual reproduction. What functions do petals have? (1) You have seen bees and other insects visiting flowers. What do the insects get from flowers? (2)

Now let us review the structure of the essential parts of a flower by stripping the sepals and petals away. You will find a central stalk-like body surrounded by five to ten delicate threads, each ending in a little sac. The small sacs are anthers in which thousands of tiny pollen grains are produced. Each anther, with its thread, is a stamen, and these make up the male parts of a flower. The number of stamens varies according to the kind of flower. What is the number of stamens on the flower you are using? (3) What are some of the ways in which pollen is carried from the anthers to the female part of a flower? (4)

The central stalk surrounded by the stamens is the female part of the flower, called a pistil. It is composed of an enlarged basal part, the ovary, above which is an elongated style ending in the stigma. The stigma may have a sticky or hairy surface which traps the pollen grains and provides them with a place to grow.

With a very sharp razor blade cut the ovary lengthwise. Using a hand lens or dissecting microscope look at the cut surface. How many ovules can you see inside the ovary? (5) Each one of these ovules contains an **egg cell**. How do you suppose the egg is fertilized when it is so well protected inside the ovary? (6) How close to the egg can a pollen grain get? (7) If the pollen grain cannot get to the egg directly, how do you suppose the sperm cells produced by the pollen reach the egg? (8)

The union of egg and sperm causes extensive changes in the female reproductive parts. Fertilization of the egg stimulates the growth of the ovary and the enclosed ovules. Examine some fresh pea pods or beans. What part of the female reproductive apparatus is the pod of a bean or pea? (9) Open up the pod and you will find the **seeds**. What is the origin of a seed? (10) If we plant ripe bean or pea seeds and water the seeds, to what will they give rise? (11) What can we conclude develops within a seed as a result of fertilization? (12)

A SIMPLE KEY TO

FLOWERING PLANTS

Using keys in identifying living things is much like traveling a road where road intersections are well marked. Roads (or choices) in the key are arranged as conveniently as possible, with no stop signs or detours until the destination is reached. In this case, the destination is the name of the family of the plant which you are keying.

■ The purpose of this exercise is to emphasize important differences in a general structural pattern, and to show how these differences may be used in identification and classification.

MATERIALS

Representatives of common families of flowering plants
Stereoscopic dissecting microscope or hand lens
Scissors
Scalpel
Forceps
Dissecting needle

PROCEDURE

Use of a Key. Choose a flowering plant for identification and determine whether it belongs with the monocotyledons or dicotyledons. Consult the two pages of keys (Figures 17-2-1 and 17-2-2). After having decided that your plant belongs to one of the two major groupings of flowering plants, choose the correct one of the two alternatives, indicated by arrows on the chart. In the key, the road signs you are to follow are not names of towns or route numbers but *characteristics* of the plant you wish to identify as to family. Some questions you will need to ask and answer are:

1. How many sepals, petals, stamens, and carpels are there?
2. Are these structures separate or united?
3. Is the flower regular or irregular?
4. Is the ovary superior or inferior?
5. Are the carpels united or separate?
6. Are petals and other floral parts present or absent?

If any of the terms used are unfamiliar, consult the following glossary of terms and the illustrations in Figure 17-2-3. Practice in observation, understanding of terms, and care in choosing the right road will soon enable you to find the right family to which your plant belongs.

GLOSSARY OF TERMS

(Numbers of definitions correspond to numbers in Figure 17-2-3.)
1. **Superior ovary:** ovary located above the points of attachment of the sepals and other floral parts.
2. **Inferior ovary:** ovary located below the points of attachment of the sepals and other floral parts.
3. **Irregular flowers:** flowers in which one or more members of a particular whorl of floral parts (sepals, petals, or stamens) are dissimilar in shape.
4. **Regular flowers:** flowers whose petals (sepals or stamens) of similar shape and size are usually equidistant from each other.
5. **Petals united:** the condition in which some or all petals are totally united or at least partially united at the base. The corolla lobes may still be separate. A corolla of united petals may be in the form of a bell, a tube, a strap, or a funnel.
6. **Petals separate:** petals separated as pictured.
7. **Carpels separate and many:** as in many members of the rose, buttercup, and water
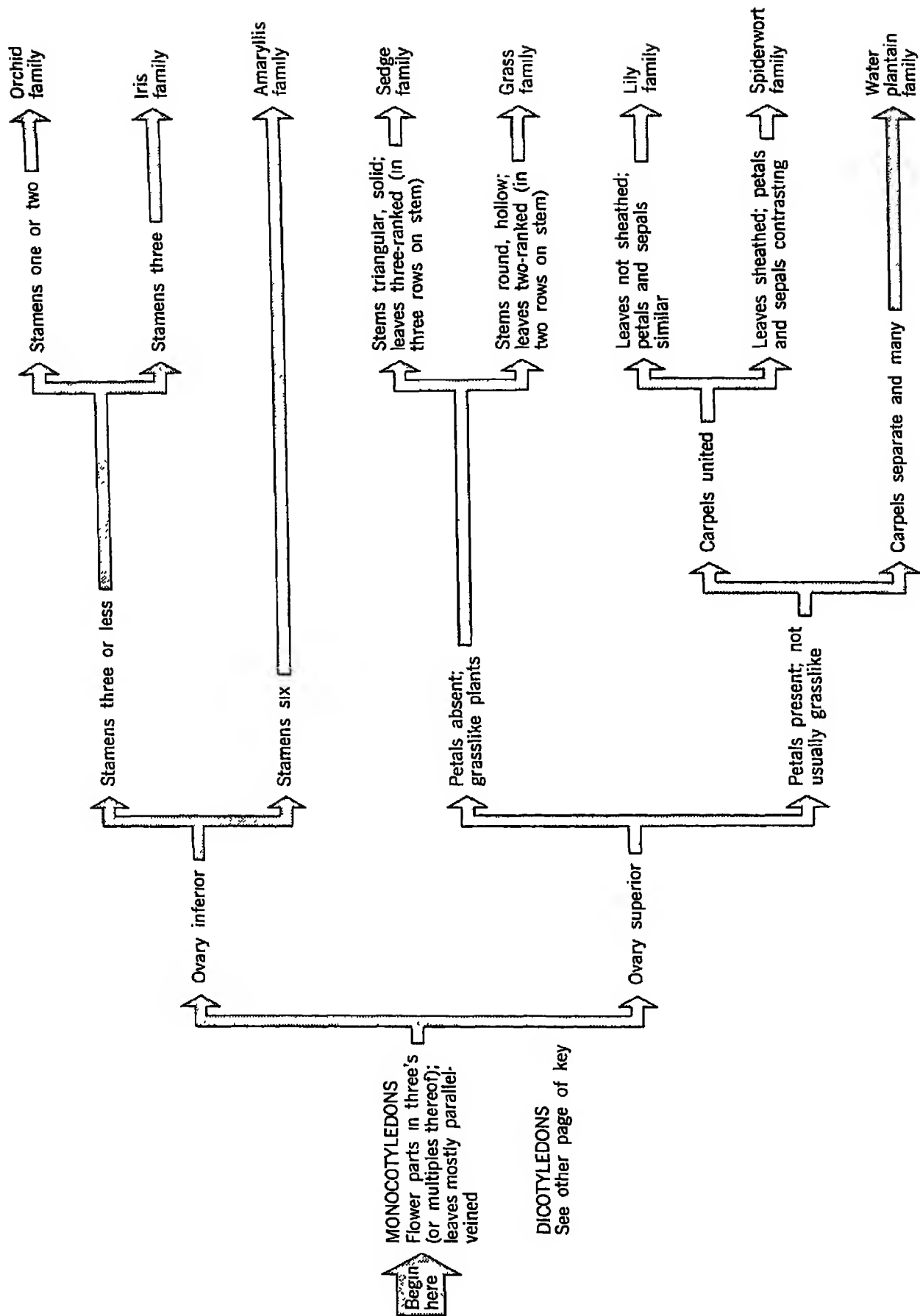
Figure 17-2-1 Key to monocotyledons.
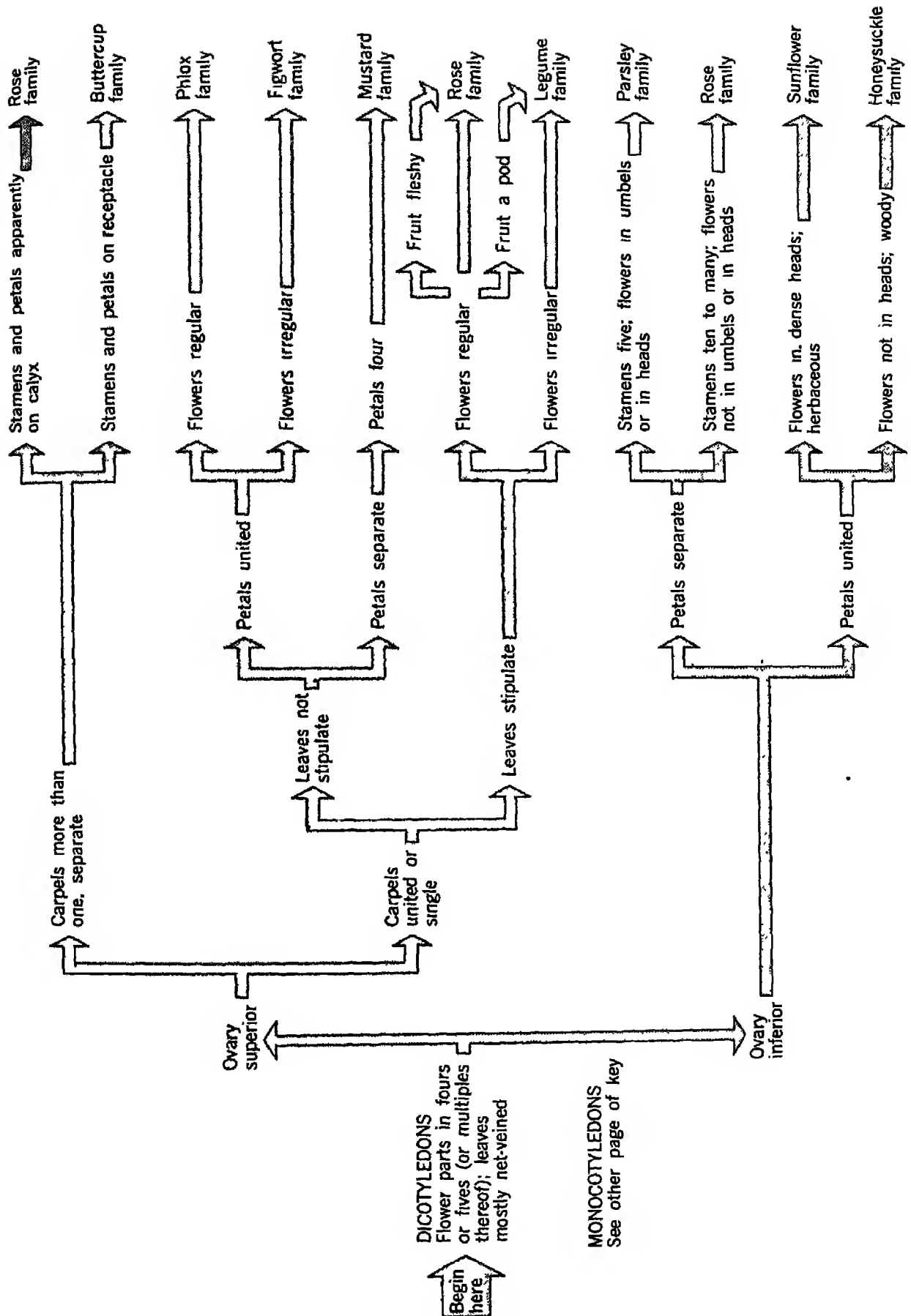
Figure 17-2-2 Key to dicotyledons.

1. Superior ovary

2. Inferior ovary

3. Irregular flower

4. Regular flower

5. Petals united

6. Petals separate

7. Carpels separate and many

8. Carpels united

9. Head

10. Umbel

11. Stipules

12. Sheath
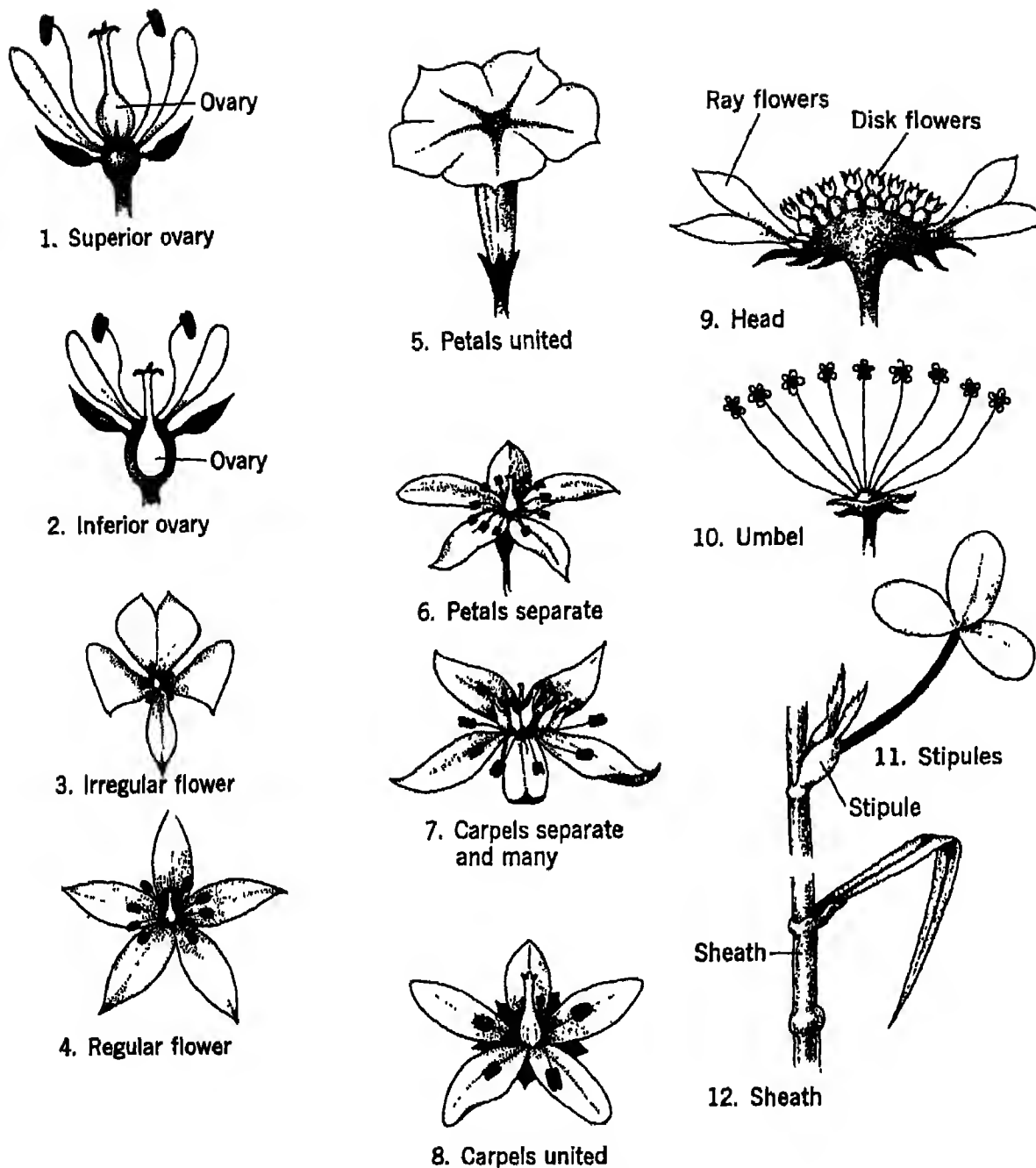
Figure 17-2-3 Illustrated glossary of flower terminology.

plantain families, there may be many separate carpels in a flower.

8. **Carpels united:** A pistil may be the product of fusion of two or more carpels. The number of carpels, when united, is frequently indicated by the number of lobes of the ovary, the number of styles, or lobes of the stigma.

9. **Head:** an inflorescence in which many flowers are sessile (without floral stalks) and grouped closely on the receptacle. Flowers such as those of the daisy or sunflower are arranged in this manner. Petal-like ray flowers are irregular with petals united into a flat, straplike "ray." The disk flowers are regular and have the petals united in a tubelike structure.

10. **Umbel:** an inflorescence in which the individual floral stalks arise from the top of the main stem of the inflorescence.

11. **Stipule:** a leaflike or sometimes spinelike structure attached at both sides of the base of the leaf or petiole. Stipules are conspicuous in roses and legumes.

12. **Sheath:** the basal part of a leaf which wraps around the stem.

After you have found the family to which your plant belongs, confirm your conclusion by reading the additional description of the families, given below.

Monocotyledons

Amaryllis Family: herbaceous plants with 3 petals and 3 sepals, separate or united, and similarly colored; stamens 6; ovary inferior; carpels 3, united. Much like a lily except for the inferior ovary.

Iris Family: herbaceous plants; 3 sepals and 3 petals both brightly colored; flowers regular or irregular; stamens 3; ovary inferior.

Grass Family: herbaceous annuals or perennials; leaves usually narrow and composed of a sheath enclosing the stem and a free portion called the blade; flowers minute, surrounded by chaffy bracts; without showy petals and sepals, flowers in spikes or spikelets.

Lily Family: same description as for amaryllis family except that members of the lily family have a superior ovary.

Sedge Family: grasslike plants with sheaths united around the stem; flowers perfect or imperfect, with 1 or 2 scales below; stamens 1 to 3; ovary superior, sometimes surrounded by a saclike structure; without showy sepals and petals.

Water Plantain Family: herbaceous aquatic or marsh plants; leaves usually broad, petioled, with sheathed bases; 3 sepals, 3 petals, separate; carpels, several to many, separate; ovaries superior.

Spiderwort Family: herbaceous, somewhat succulent plants; leaves alternate with sheathed bases; 3 persistent and usually green sepals; 3 petals, separate, usually colored and quickly withering; ovary superior; stamens 6.

Orchid Family: perennial herbaceous plants, a few without chlorophyll; ovary inferior; flowers irregular; lower of 3 petals modified to form a liplike or saclike structure; stamens 1 or 2, united with the style to form a column.

Dicotyledons

Buttercup Family: herbaceous plants; petals 5 or more, separate, usually yellow or white; stamens 10 or more; pistils 5 to many; ovaries superior. This is a primitive family of flowering plants.

Composite Family: flowers arranged in a head generally like those of dandelion, daisy, or chrysanthemum; occasionally like the goldenrod; petals united, ovary inferior; stamens usually 5, attached to the corolla and united by the anthers. This family has highly evolved flowers.

Honeysuckle Family: shrubs or vines with opposite leaves, without stipules; flowers regular or irregular; petals united; stamens 5, inserted on the lobes of the petals; ovary inferior.

Legume Family: flowers usually irregular; leaves usually compound; fruit a legume (pealike pod); herbaceous plants, shrubs, or trees; stamens 4 to 10, commonly with 9 united by their filaments, 1 free or nearly so.

Mint Family: leaves opposite; stems usually square in cross section, often aromatic; flowers irregular; 5 sepals, united, commonly 2-lipped; ovary superior, usually 4-lobed; fruit 4 little seedlike nutlets; stamens 4, in 2 unlike pairs.

Mustard Family: petals 4; sepals 4; ovary superior; stamens in 2 sets, 4 long and 2 short (rarely only 2 or 4); often with an odor like a turnip or radish.

Parsley Family: herbaceous plants; flowers small, generally in a simple or compound umbel; petals 5; stamens 5; leaves alternate and usually compound; ovary inferior.

Phlox Family: herbaceous plants; flowers perfect, regular; 5 united petals; stamens 5, filaments attached to the united petals; ovary superior; styles dividing into 3 linear stigmas.

Rose Family: herbaceous plants, shrubs, trees; sepals and many stamens in a ring surrounding the carpels; leaves usually with stipules; flowers regular, usually perfect; ovaries 1 to many, superior or inferior.

SEEDS AND HOW THEY GROW

Because bean seeds are easy to get, are large, and show the parts of a seed very well, we will use them for our observations.

■ The purpose of this exercise is to study the structure of a dicotyledonous seed and its subsequent germination.

> **MATERIALS (Parts A and B)**
>
> Soaked bean seeds
> Germinating bean seeds (3 days old)
> Germinating bean seeds (10 days old)
> Stereoscopic dissecting microscope
> Iodine solution, dilute

PROCEDURE
Part A:
The Seed

First we want you to see some of the external features of a seed; then we will look at the inside. It is obvious that the seed is covered by a tough leathery coat. How do you think this coat functions? (1) Look along one edge of the seed and you will find an elliptical scar. This scar represents the place where the seed was attached to the parent plant—through which the developing seed obtained its nutrition. Do you have an analogous scar on your body? (2) In what stage of development were you when you were attached to your mother? (3) This same stage is represented by structures inside the seed coat.

Remove the seed coat. Inside of the seed coat you will find the **embryo**. There are two fleshy halves called **cotyledons** which make up part of the embryo. These two halves are the same structures found in peanuts, peas, and many other kinds of seeds. Cut a little sliver from one of the halves (do not touch the part that has the little plant remaining attached to it) and test it with iodine solution. What

would you deduce is the primary function of the cotyledons? (4) What was the original source of the food in the cotyledons? (5)

Find the little plant attached to one end of the cotyledons. This is the rest of the embryo. Take a closer look at it with a hand lens or dissecting microscope. You will see that this part of the embryo has two miniature leaves and a rootlike portion. The small leaves plus a tiny shoot tip make up the epicotyl of the embryo, the root portion the hypocotyl. Observation of stages in seedling growth will help us to understand what happens to the various parts.

Make your own drawing showing the parts of a seed that were visible when the seed was split open.

Part B:
The Seedling

Bean seeds germinate and grow rapidly, and this makes it possible for us to compare 3-day-old and 10-day-old stages. We think you will agree that some rather remarkable changes take place during 10 days of growth in a plant, when you remember what the seed that gave rise to the plant looked like.

Look at 3-day-old seedlings in the soil before your teacher digs them out for closer observation. What mechanism does the plant have for clearing a path through the soil? (6) What part of the plant becomes established first? (7) What are the functions of the root? (8) What part of the embryo gives rise to the root of a bean plant? (9) Where are the first leaves of the seedling? (10) What part of the embryo produces the first leaves? (11)

Compare a 10-day-old seedling with one 3 days old. Are the cotyledons still present? (12) Where is the seed coat? (13) Find the stem of the plant. What part or parts of the embryo developed into the stem? (14) How are the first two miniature leaves arranged on the stem? (15)

PLANT REACTIONS

TO ENVIRONMENT

Many people have the idea that because most plants are anchored they are incapable of reacting to favorable or unfavorable conditions. Although animals, including man, can react in a spectacular way to changes in environment, for example, reaction to a stimulus causing pain, plants in more subtle ways respond to various environmental factors.

■ The purpose of this exercise is to determine how a seed manages to send the shoot up and the root down, and to determine the effect of light on the growth of plants.

MATERIALS

Part A

Six Petri dishes (150 x 20 mm)
Soaked corn grains
Blotting paper or filter paper
Cotton
Wax pencil
Cellophane tape

Part B

Four boxes, three of them constructed to provide one-sided illumination
Blue and red cellophane
Four flowerpots planted with radish seeds
Three artificial lights

Part A:

Response to Gravity

At one time or another you must have wondered if it was necessary to plant a seed or grain right-side-up so that the root would grow down into the soil and the shoot up through the soil into the light. We may have seen farmers planting seed and grains with a machine called a drill. The drill makes a narrow furrow

into which the seeds are dropped, then covered. It is obvious that the machine does not orient the seeds of grains.

PROCEDURE

Select four plump, soaked corn grains and place them on the bottom of the empty Petri dish; place the four grains horizontally with their pointed ends directed toward the center, one grain at each point of the compass (north, south, east, west). (See Figure 17-4-1.)

Cut a piece of blotting paper to fit tightly inside the bottom half of a Petri dish. Cover the corn grains in the dish with the paper. Pack the rest of the dish tightly with cotton so that when the cover is placed on the dish and the dish is set up on edge, the corn grains will stay in place and show through the bottom of the dish.

When you are sure that the grains will stay in place, open the dish and wet the blotting paper. Close the dish and seal it with strips of tape. Put the dish on edge with one grain at the top position. Mark the dish with the word TOP and let the grains germinate *without* changing the position of the plate. If you leave the plate in this position you will have one corn grain germinating in the "normal" position, two germinating from a horizontal position, and one upside-down.

It will take about 3 days for the grains to germinate. In what directions do the root and shoot grow out from each of the grains? (1) To what factor of the environment do the root and shoot respond? (2)

Part B:

Response to Light

The bending of plants toward light is one of the most common reactions of plants to their environment. It is a phenomenon readily seen
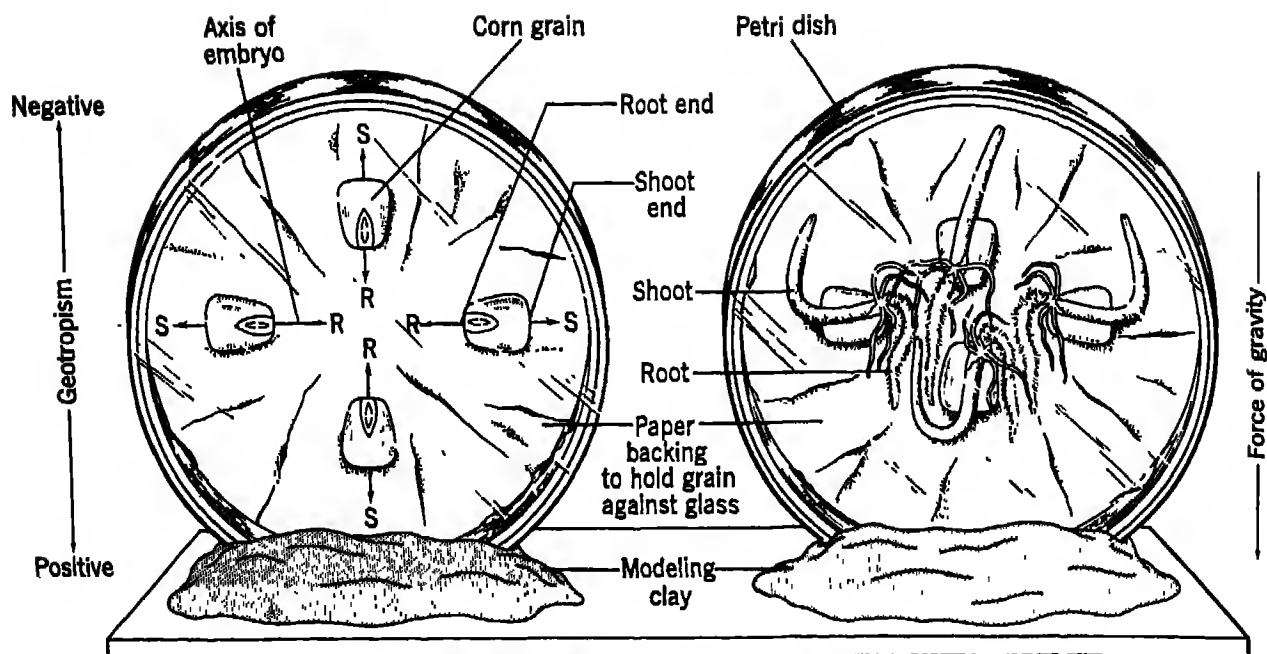
Figure 17-4-1   Geotropism in sprouting corn grains.

in nature and in our daily lives. For example, when we place plants in a window, their stems and leaves bend toward the light. Sunflowers are especially noted for turning toward the light of the sun (heliotropism).

PROCEDURE

Plant and water four pots of radish seeds and place each one directly into one of the boxes. Prepare the boxes as follows:

Box 1. Seal this box to make it light-tight.
Box 2. Let the opening on this box remain uncovered so that the seedlings will receive one-sided illumination from white light.
Box 3. Cover the opening of this box with the red cellophane.
Box 4. Cover the opening of this box with blue cellophane.

After about 5 days under constant illumination, the radish seedlings will be ready for observation. Do not take the pots out of the boxes or move them until the observations are completed.

Compare the plants in the three boxes with one-sided illumination. Which ones show the most obvious change in direction of growth? (3) Is there any difference in the reactions of the plant to blue light and to red light? (4) Are these two wavelengths of light equally effective in stimulating plants to bend toward light? (5)

Compare the size and color of the plants (all planted at the same time) in the boxes receiving no light and white light. What is the general effect on stem elongation of white light versus no light? (6) How might you account for this difference? (7)

Compare the size and color of the plants (all planted at the same time) in the boxes receiving red light and blue light. What is the general effect of red light versus blue light on stem elongation? (8)

REGULATION OF

GROWTH IN PLANTS

In previous experiments you have observed evidence that plants adjust to some factors of their environment. A common example is the bending of plants toward light. This happens, it is often said, "because the plant needs the light" in order to live and grow. Do you believe that plants grow toward the light because they need to do so? In one sense plants do "need" to grow toward the light, for they would soon die without it. But we must be careful to recognize that the plant does not purposefully turn toward the light. In this exercise we shall be concerned with the question: How can we explain the growth of plants toward the light?

The mechanisms of this and other responses such as those to gravity, water, and solutes, have been studied extensively. Critical experiments to study such responses require carefully controlled conditions of temperature, humidity, and light; uniform plant material; and the necessary skill in handling the material. For our purposes it will be more convenient and practical to describe, in some detail, some of the experiments which have been carried out and to provide you with the results. You are to suppose, then that you are a scientist who has performed the experiments and obtained the results given.

■ The purpose of this exercise is to analyze the given experimental data and to draw conclusions from them regarding the role of auxins (hormones) in the regulation of growth in plants. This is also an important activity of the scientist.

MATERIALS: None

First, you will need the following information:

Five experiments will be described. In all of the experiments, groups of oat seedlings have been used as the experimental material. The results were essentially the same for all plants in each group, so only one plant for each group will be described. All plants were grown under the same constant temperature and constant humidity.

You will recall that the tip of a stem contains tissue which is composed of rapidly dividing cells. This is the meristem. The region below the tip is the region of elongation, where cells elongate but do not divide.

The shoot of an oat seedling is covered by an outer sheath, the coleoptile, which covers the developing embryo leaves. When the shoot reaches a length of 5 to 8 cm, the coleoptile ceases to grow. The enclosed leaves split through the coleoptile and continue their growth upward.

The coleoptile, like a typical stem, has a tissue composed of rapidly dividing cells at the tip with a region of elongation below the tip. In the following experiments we will be concerned only with the coleoptile of the seedlings.

Agar is a product of seaweeds. It will dissolve in hot water. When agar cools in solution it resembles gelatin. Substances soluble in water readily diffuse through agar.

Experiment 1

Three groups of oat seedlings of the same size (A, B, and C representative of the plants in each group) were used for this experiment. Plant A was untreated. The tip of meristem tissue was removed from Plant B. The tip was then replaced on the cut surface of the plant. The tip was cut off Plant C and was discarded. *All plants were grown in the dark.* (See Figure 17-5-1.)

EXPERIMENT 1

Control

Tip cut off
and replaced

Tip removed

10 Hours
later

A   B   C   A   B   C   A   B   C
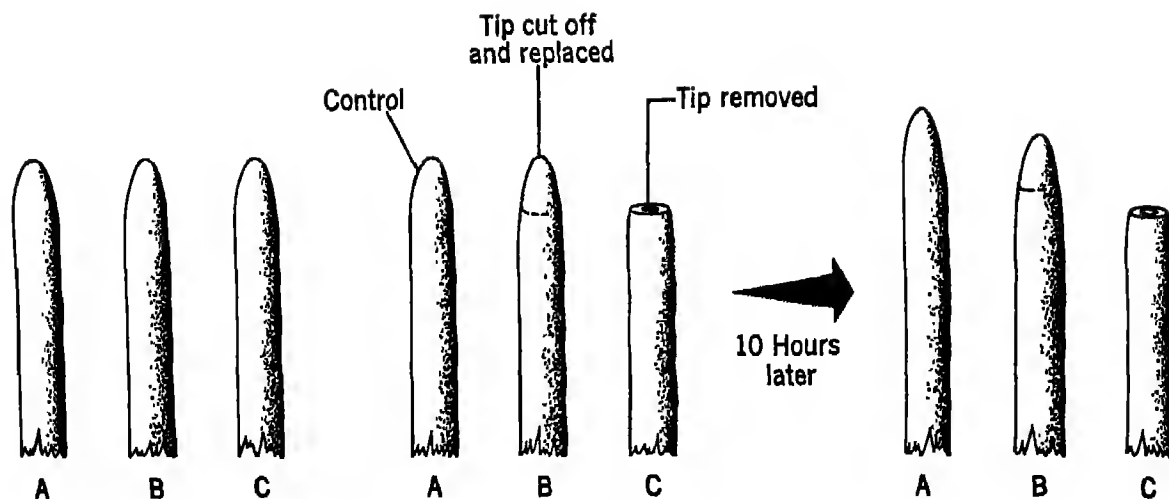
Figure 17-5-1

EXPERIMENT 2

Agar block   Mica

D   E   F   D   E   F
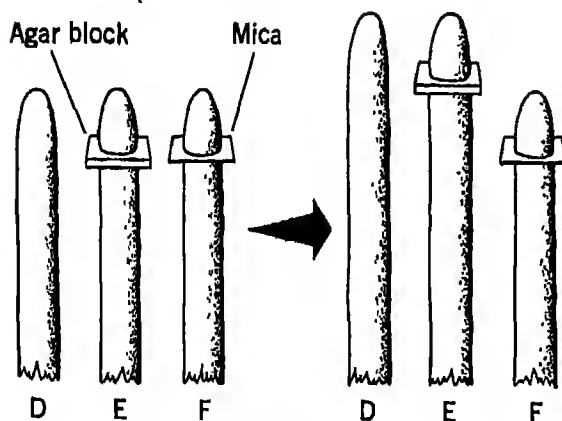
Figure 17-5-2

EXPERIMENT 3

Agar block
containing
juice   Plain agar
block

G   H   J   G   H   J
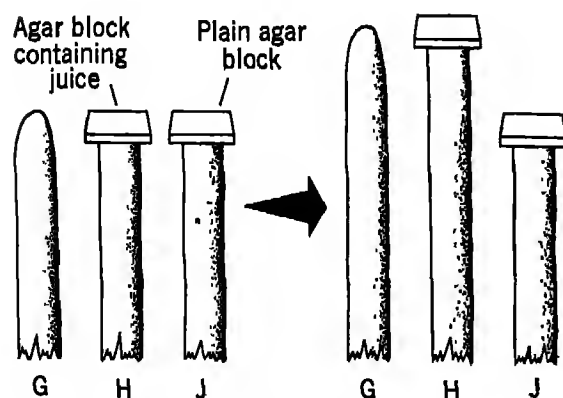
Figure 17-5-3

Plant A grew normally and when the experiment ended, this plant was one-third taller than it had been at the beginning. Plant B was only slightly shorter than A. Plant C was of the same height as at the beginning of the experiment.

According to what has been said, does the coleoptile elongate above the cut or below the cut? (1) What evidence is there that some stimulation from the tip is necessary for elongation of the coleoptile? (2) How do the two experimental plants differ from each other at the beginning of the experiment? (3) Why was Plant A included in the experiment? (4) What conclusion can you draw from the results of this experiment? (5)

Experiment 2

Three more groups of oat seedlings, represented by D, E, and F, were used in this experiment. Plant D was untreated. The tip was cut off Plant E and a block of agar 1 mm thick was placed on the top of the cut coleoptile. The tip was then replaced on top of the agar. The tip was also removed from Plant F, and a thin sheet of mica (a mineral substance that prevents diffusion) was placed between the coleoptile and tip. *All plants were grown in the dark.* (See Figure 17-5-2.)

Plant D grew normally. The coleoptile of Plant E also grew normally. Plant F did not grow.

How do the two experimental plants differ from each other at the beginning of the experiment? (6) Why was Plant D included in this experiment? (7) What new information is gained as a result of this experiment? (8)

Experiment 3

The juice was extracted from tips of oat seedlings and mixed with agar, from which small agar blocks were made. Blocks were also made of plain agar. Three groups of oat plants were again used, G, H, and J. Plant G was untreated. A block of agar containing juice from growing tips was put on top of the coleoptile of Plant H, from which the tip had been removed. A plain agar block without the juice of the tips was placed on top of the coleoptile of Plant J, which also had the tip removed. *All plants were grown in the dark.* (See Figure 17-5-3.)

Plant G grew normally. Plant H also grew normally. Plant J did not grow at all.

How do the two experimental plants differ from each other? (9) Why was a plain agar block used on one of the plants? (10) Is the coleoptile tip itself required for elongation of the coleoptile or only the substance produced in the coleoptile? Explain. (11)

Experiment 4

Three plants (K, L, and M) from Experiment 4 are shown in Figure 17-5-4. Plant K was allowed to grow normally. The tips (except for the center core of leaves) were removed from Plants L and M. A block of agar mixed with the juice from growing tips was placed on the left half of the cut surface of the stump of Plant L, and a piece of agar treated in the same way was placed on the right half of the stump of Plant M. *All plants were grown in the dark.* (See Figure 17-5-4.)

Plant K grew normally. Plant L curved to the right. Plant M curved to the left.

Where did most of the growth (elongation of cells) take place in Plant L? (12) In Plant M? (13) How do you account for the different rates of growth on the left and right sides of the experimental plants? (14) Recall that these plants were grown in the dark. See if you can suggest, on the basis of these results, a mechanism that would explain the fact that growing plants turn toward the light. (15)

Experiment 5

Again three plants, N, O, and P, will be considered. Plant N was untreated. A very small piece of mica was inserted between the growing tip and the rest of the coleoptile on the left side of Plant O; and a piece of mica was inserted into the right side of Plant P. *The tips of all three plants were illuminated from the right side only.* (See Figure 17-5-5.)

Results. Plant N grew and curved to the

EXPERIMENT 4
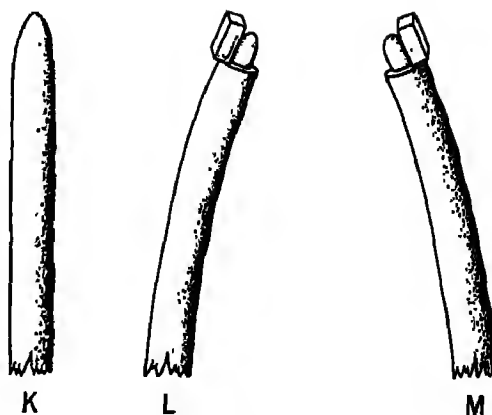
Figure 17-5-4

EXPERIMENT 5
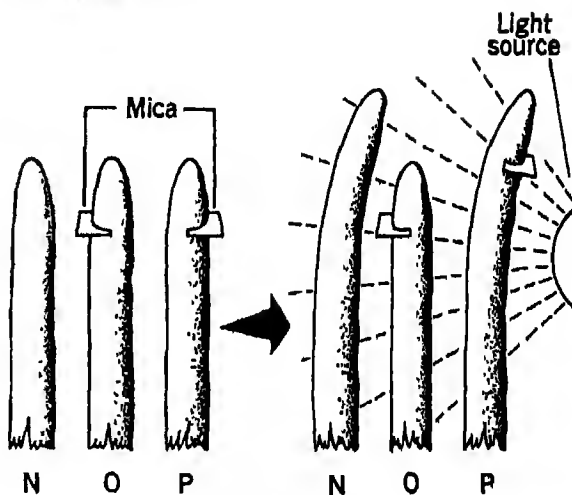
Figure 17-5-5

right. Plant O failed to grow. Plant P grew and curved to the right.

Recall the results of the first four experiments, as well as those of Experiment 5. On the basis of all these you should be able to provide possible answers to the following questions.

What caused elongation? (16) Under what conditions of illumination were the first four experiments performed? (17) What seems to be the effect of light in Experiment 5? (18) Why does elongation take place on one side only in Experiments 4 and 5? (19) Explain why Plant O failed to grow. (20) Now go back to the question in the introduction, "Do you believe that plants grow toward the light because they need to do so?" (21) Explain *how* the growing plant bends toward the light. (22)

Animals

The following exercises will introduce you to the animal way of life. Here you will find organisms which are coordinated and have the ability to move freely, and which demonstrate a complex type of behavior. These organisms have adapted not only to an environment, but also to the necessity of obtaining food. In this last adaptation, animals, which are unable to make their own food, provide a marked contrast to plants, which are able to build complex carbohydrates from simple molecules.

In the first six exercises, you will be asked to work like a scientist and make observations, form hypotheses, and perform experiments to test them. The result of this series of experiments will be an understanding of the animal as a functioning organism. We use as our typical animal the simple one-celled *Paramecium* which dramatically shows all of the major processes involved in the animal way of life.

In the exercises to accompany Chapter 20, you will be introduced to a variety of multicellular animals and will be asked to compare and contrast their structures and functions. In this section, as in other sections of the laboratory manual, we are less concerned with names of structures and animals than we are with understanding general patterns of organization and the functioning of animals in environments to which they have become adapted.

We will deal at some length with the various processes common to multicellular animals in order for you to understand more fully the basic characteristics of all animals and the modifications which distinguish one from another.

As you do these exercises, keep in mind not only the ways in which animals and plants differ, but also the structures and functions they have in common. The structures and functions that occur again and again in our study of cells, microorganisms, plants, and animals, will serve to clarify your understanding of what life is. The ways in which animals differ from other organisms studied will emphasize the great variety of forms in which life exists on this earth.

STRUCTURE AND FUNCTION

IN *PARAMECIUM*

Earlier in this course we inquired into the basic common nature of all living organisms. We asked the questions: What does being alive actually mean? How does a living organism differ from a dead one? Now let's ask the question: In what general ways do individual organisms perform the functions of living?

Every organism is a variation on structural and functional themes created many millions of years ago. Although the living world today is a varied collection of unicellular and multicellular organisms, most organisms perform the same basic activities. Thus, the study of one animal teaches us something about many animals.

We shall concern ourselves, for the present, with the animal way of life. By closely examining the ways in which a single animal performs its life functions we can come to a better understanding of how the great variety of animals meet their problems of living. We do not need a walking, stalking beast for this purpose. The unicellular organism *Paramecium*, which was probably seen in Exercise 3-7 when pond water was examined, carries out all metabolic and reproductive functions within the confines of one small cell. For this and other reasons, *Paramecium* is a convenient organism for our study of the way of life of animals in general.

In Exercises 19-1 through 19-6 you will study *Paramecium* in greater detail, not simply observing it as you did in Exercise 3-7. You will see it move, ingest and digest food, get rid of waste materials, respond to its environment, and reproduce its kind. There are other life functions which cannot be seen as easily as these, but you may read about them in your textbook.

■ The purpose of this exercise is to examine a typical animal (*Paramecium*) and to begin to correlate the functions of living with the structures observed.

MATERIALS

Culture of *Paramecium*
Compound microscope
Methyl cellulose solution
Iodine or methylene blue stain
Pipettes for transfer of organisms and solutions
Slide and cover glass

PROCEDURE

With a pipette containing several drops of syrupy methyl cellulose solution, make a small ring (about the diameter of a lead pencil) in the center of a clean slide. With a second pipette place one drop of *Paramecium* culture on the slide inside the ring and add a cover glass. Examine the drop carefully with the low power of your microscope. Use high power only for detail. What is the general shape of the animal? (1) Do the paramecia move slowly or rapidly? (2) Movement will become slower as the methyl cellulose solution diffuses toward the center of the drop.

Draw an outline of *Paramecium* similar to Figure 19-1-1 and include in your drawing all the parts that you observe. What structures are in Figure 19-1-1 that you cannot see? (3)

Each of the structures in *Paramecium* is performing some function necessary for its life. For the purposes of this exercise you are to imagine you are a biologist who is observing an unknown organism for the first time. By carefully observing *Paramecium* and its structure you are to form a series of hypotheses regarding the function of each structure you see. You can formalize these hypotheses as a series of statements, such as, "I believe that structure —— is concerned with digestion of food." Number your hypotheses according to the following scheme: 1. The structure that serves for

food intake. 2. The structure that is concerned with digestion of food. 3. The structure that acts to discharge undigested food remains. 4. The structure that serves for oxygen and carbon dioxide exchange. 5. The structure that moves the animal from place to place. 6. The structure that serves to excrete fluid wastes. 7. The structure that serves as a hydrostatic organ (organ to remove excess water).

Using these same numbers, number the structures on Figure 19-1-1 in accordance with what you believe their function to be. Obviously, you will not be sure you have labeled the structures correctly until we finish our study of *Paramecium*. However, use your imagination and your eyes and make the best possible observations for your hypotheses. Do not worry at this time about the names of the parts you have labeled but consider only their functions.

After you have finished your observations of the living *Paramecium*, add a drop of iodine or methylene blue stain to the slide. What structures can you see now that you did not see in your previous observations? (4) Add these structures to your diagram, darkening them in so that they will be distinguished from the structures you saw in the living organism. Are any of the structures you are able to observe in the living organism now no longer visible? (5) For the purposes of forming the hypotheses above, which gave you the most information, the living organism or the dead stained one? (6)

What functions of a living organism are not represented in the seven hypotheses above? (7) If this were a plant cell instead of an animal cell, would you expect to observe all the structures you have labeled in relation to your seven hypotheses? (8)

For the moment assume that all of your hypotheses are correct. What conclusion could you then draw about the functions of the typical animal? (9)
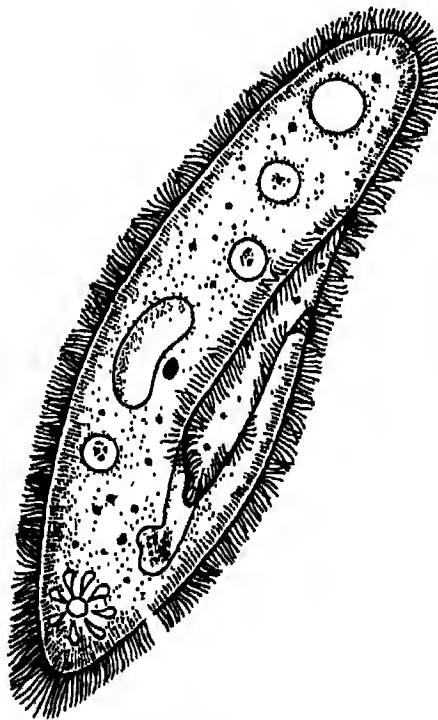
Figure 19-1-1

MOVEMENT

AND *PARAMECIUM*

One easily observable characteristic of animals is movement. Frequently we contrast plants and animals on the basis of their ability to move or their lack of such ability. However, not all animals can move freely. An animal that is fixed to its environment is said to be sessile. Can you think of any sessile animals? (1)

The *Paramecium* is a rapidly moving protozoan.

■ The purpose of this exercise is to attempt to find patterns and methods of movement in *Paramecium.*

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MATERIALS

Paramecium culture
Methyl cellulose solution
Carmine solution
Cotton fibers
Compound microscope
Slide and cover glass
Pipettes
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PROCEDURE

Make a thin ring of methyl cellulose (about the diameter of a lead pencil) on a clean slide and add a drop of *Paramecium* culture. Cover with the cover glass and observe first under the low power of your microscope.

Before the methyl cellulose has diffused into the water the paramecia will move very rapidly. When they begin to slow down, switch to high power to observe motion in detail.

Is the same end of the paramecium usually in front as the paramecium moves? (2) The front end of an animal is referred to as its anterior. The back end is referred to as the posterior. On Figure 19-2-1, on the following page, label on the basis of your observation what you believe to be the anterior and posterior ends of the paramecium. Do these two ends vary in shape? (3) On the basis of your observation why do you believe *Paramecium* has been called the "slipper animalcule"? (4) Does the shape of the paramecium vary as the animal moves? (5) Is there a definite top and bottom to the animal as it moves? (6) Describe the motion of the paramecium as it moves in one direction. (7) By means of arrows show the directions of the motions of the body as it moves forward. Use Figure 19-2-1 for this purpose also.

Add a few cotton fibers to your *Paramecium* slide and notice carefully the reaction when the paramecium bumps into one of these fibers. Describe what happens when the paramecium bumps into something. (8) Draw a line to indicate a cotton fiber. By means of arrows show the direction of movement of a paramecium which has just bumped into the cotton fiber. What do the reactions of a paramecium to an obstacle in its path suggest about its behavior? (9)

Add a small drop of carmine solution to the drop of *Paramecium* culture on the slide. Under high power observe the surface of the animal. The hairlike structures are cilia. What do you believe their function to be? (10) Notice carefully the passage of carmine particles along the surface of the paramecium. What does their pattern of movement tell you about the activities of the cilia? (11) Can you see individual cilia? (12)

Now refer back to Exercise 19-1. On the basis of your observations from this exercise formulate a hypothesis concerning the method of locomotion in *Paramecium*. (13)

In what way has a study of movement in *Paramecium* contributed to your understanding of locomotion in animals in general? (14) Cilia are found in many multicellular animals including man. However, except in such forms

as the planarian, they do not serve to move the entire organism, but rather are found localized in such regions as the pharynx of the frog, the trachea of man and other internal passages.
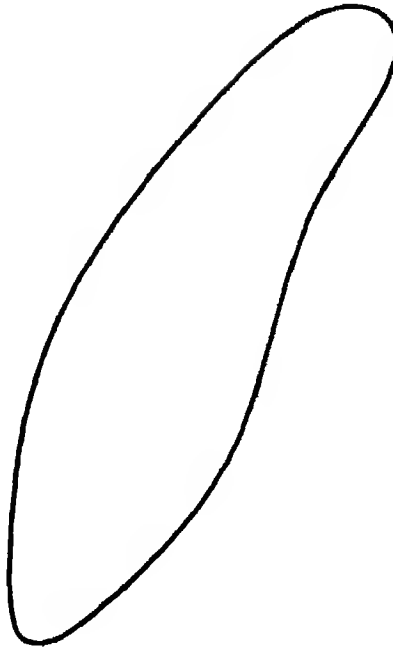
Figure 19-2-1

INGESTION AND DIGESTION IN *PARAMECIUM*

Animals differ from plants in being heterotrophic. That is, they are unable to make the basic chemical nutrients they require, and thus must prey on other animals or plants for the basic chemicals necessary for their metabolism. *Paramecium* is no exception.

■ The purpose of this exercise is to observe *Paramecium* eating small plant cells (yeast) and to learn how digestion takes place to obtain the essential food requirements.

MATERIALS

Paramecium culture
Yeast suspension
Congo red solution
Methyl cellulose
Microscope
Slide and cover glass
Pipettes

PROCEDURE

Before Laboratory Period. Mix half a cake of yeast in 50 ml of water and let the mixture set for about 24 hours. Then boil it, and add 0.1 g of Congo red powder. Keep the mixture close to boiling for about 8 minutes, but be careful not to burn the yeast by too high a temperature. Allow the mixture to cool.

During Laboratory Period. Prepare a ring of methyl cellulose as in Exercise 19-1. Place a small drop of *Paramecium* culture in the center of the slide and add a drop of the yeast suspension. Cover and immediately observe it under the microscope. It will take only about 10 seconds for each paramecium to ingest (eat) some of the yeast cells. What color are the yeast cells? (1) What is the source of the color of the yeast cells? (2) Watch the colored yeast

cells being ingested. Where do they enter the body of the paramecium? (3) Notice that they are enclosed by a tiny droplet to form a food vacuole. Where are the food vacuoles formed? (4) Carefully follow the course of a food vacuole. Within 2 or 3 minutes you should begin to notice a change in color in the food vacuole. What is the color of the vacuole after 2 or 3 minutes? (5) As you continue to watch, are there any further color changes in the food vacuoles? (6)

Congo red is an indicator which changes color as the pH changes. Review the effects of an acid or base on enzyme action in Exercise 6-6. On the basis of what was learned in Exercise 6-6 and your observations now, make a statement about the significance of the pH on changes (digestion) observed in the food vacuoles of *Paramecium*. (7)

Trace the passage of the food vacuoles as they start circulating slowly in the cytoplasm. By means of arrows, and circles representing food vacuoles, show on the accompanying diagram (Figure 19-3-1) the path the food vacuole takes from its formation to its emptying to the outside. How do the food vacuoles move through the cytoplasm? (8) Compare this action with similar activity observed earlier in plant cells.

Notice carefully to see if undigested food residues are being discarded and ejected (or egested) to the outside of the animal. Is this accomplished all over the surface of the paramecium? (9)

Refer to Exercise 19-1, in which you formulated your hypothesis regarding structures responsible for food digestion and the discharge of undigested food. On the basis of the observations just concluded, summarize how you now believe food to be ingested and digested by *Paramecium*. (10)

In this exercise, of course, it was not possible to observe that paramecia are selective

in choosing their food. However, they will take in some foods while rejecting others. This selectivity is evidence of some means of test- ing food items. The ability to select between food items is apparently a primitive hetero- trophic feature.
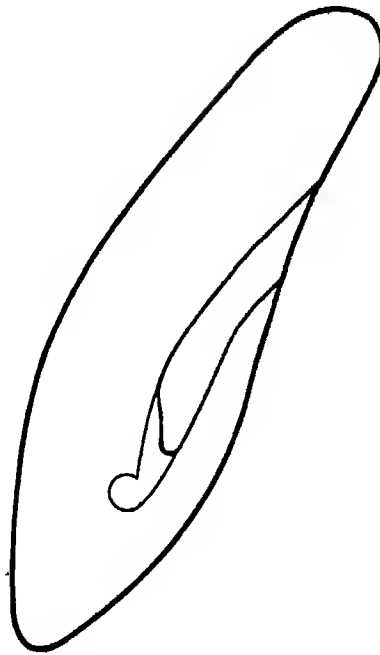
Figure 19-3-1

CONTRACTILE VACUOLES

IN *PARAMECIUM*

In observing animals one occasionally sees a peculiar structure for the first time. In *Paramecium* there is a structure known as the contractile vacuole which is not found in all free-living protozoans. The contractile vacuole fills up with fluid and contracts, expelling its contents to the outside; it then disappears. It reappears, however, again fills with fluid, and repeats this process. Sometimes it is surrounded by canals leading into it which give it a star-shaped appearance.

■ The purpose of this exercise is to investigate the appearance of the contractile vacuole and to determine its function.

MATERIALS

Paramecium culture
Microscope
Slide and cover glass
Methyl cellulose solution
Pipettes

PROCEDURE

Make a ring of methyl cellulose on a clean slide as in Exercise 19-1 and place a drop of *Paramecium* culture in its center. Add a cover glass and observe under high power until a contractile vacuole is located. How many contractile vacuoles are there in the paramecium? (1) Where are they located? (2) Draw them in place in the outline of a paramecium in Figure 19-4-1. Do the contractile vacuoles move through the body as food vacuoles do, or do they stay in one place? (3) Is the fluid within the enlarged vacuole clear or is it granular? (4) If radiating canals are seen around the contractile vacuoles, are they noticed more clearly

when the vacuole is full or empty? (5) How many radiating canals apparently empty into each contractile vacuole? (6) What is the number of contractions the vacuole makes in a period of one minute? (7)

Refer to your hypotheses in Exercise 19-1. Did you indicate that the contractile vacuole was a means of excreting wastes? (8)

Unfortunately, the techniques for proving or disproving your hypothesis require too much equipment and are too delicate to perform in your laboratory. However, you are to imagine you are a scientist trying to answer the question, "What is the function of the contractile vacuole?" For this purpose you have made a thorough search of the biological literature and have come across the following four observations which are cited here by author and journal in which they appeared in case you are fortunate enough to have a library containing them and can see the originals.

Reference 1: Urea (an end-product of nitrogen metabolism) has not been found in any significant amount in the fluid of the contractile vacuole. (Weatherby, J. H. 1927. *Biological Bulletin*, Vol. 52. Also, Weatherby, J. H. 1929. *Physiological Zoology*, Vol. 2.) On the basis of this observation would you now say that the contractile vacuole is an excretory organ, that is, that it is used exclusively for ridding the body of nitrogenous wastes? (9)

Review Exercise 6-2 and consider the evidence in the following two references.

Reference 2: If vacuoles of fresh-water protozoa do not function, the body swells. (Kitching, J. A. 1938. *Journal of Experimental Biology*, Vols. 15, 16.)

Another protozoan, *Amoeba*, has a contractile vacuole. Let us assume that it functions in the same way as the contractile vacuole of *Paramecium*.

Reference 3: Injection of distilled water into amoebae results in an increased contraction of

the vacuole. (Howland, R. B. 1924. *Journal of Experimental Zoology*, Vol. 40.)

Considering your observations in Exercise 6-2 and those of Weatherby, Kitching, and Howland, what is the most likely function of a contractile vacuole in *Paramecium*? (10)

List the ways in which water might enter the body of a paramecium. (11) What does the following reference indicate to you about the major method of water entry into the body of a paramecium? (12)

Reference 4: Water intake with food vacuoles is only a fraction of what is expelled by the contractile vacuole. (Kitching, J. A. 1939. *Biological Reviews*, Vol. 13.)

On the basis of your observations in this exercise and the four references to scientific papers, make a hypothesis that will account for the function of the contractile vacuole. (13) Suggest additional experiments which would tend to confirm or disprove your hypothesis in Question 13 above. (14)
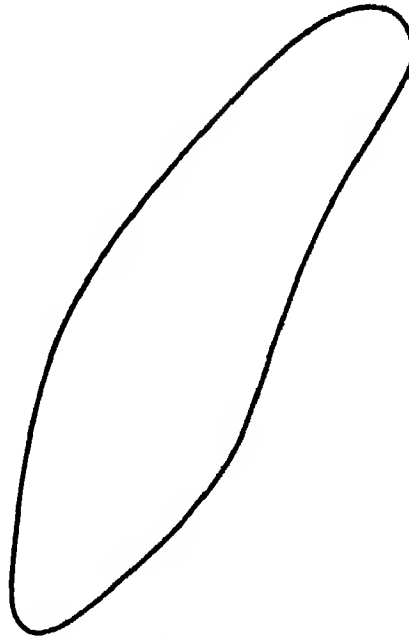
Figure 19-4-1

BEHAVIOR

OF *PARAMECIUM*

All animals respond to their environment with either instinctive or learned behavior. Even forms which lack a well-defined brain or nervous system, such as man possesses, do nevertheless have distinctive behavior patterns. In many simple animals, there is behavior of an instinctive or fixed pattern which we speak of as being a taxis. A taxis is either a direct movement in response to some stimulus in the environment or it is a series of random movements that ultimately lead the animal into appropriate conditions or favorable environments.

In higher animals and especially in man, behavior is modified by insight, the ability to learn, to remember, and similar processes whereby we possess not a fixed but a modifiable, intelligent type of behavior.

■ The purpose of this exercise is to observe the responses to the environment made by *Paramecium* and to determine what type of behavior it shows.

MATERIALS (Parts A, B, and C)

Paramecium culture

Two long vials or test tubes for each student team

Black paper or metal foil

Depression slide or small dish

Slide and cover glass

6-volt battery or four 1.5-volt dry cells connected in series, with the ends of the lead wires cleaned to act as electrodes

Reversal switch

Hydrochloric acid (HCl) solution

Cotton thread

Stopper for one of the test tubes

Compound or stereoscopic dissecting microscope

Test-tube rack

Pipettes

PROCEDURE

Part A:

Response to Gravity and Light

Pour two test tubes or vials one-quarter full of a *Paramecium* culture. Place one test tube upright in a rack or stand under even illumination.

Stopper the second test tube, and cover the top half of it with dark paper or metal foil in order to exclude light. Lay the tube on its side with the bottom half well illuminated.

Now return to observe the upright tube. In a rich culture, concentrations of paramecia may be seen with the unaided eye as a white cloud of material. Look for this concentration of paramecia. Where do the paramecia concentrate in the tube? (1) A response to gravity is spoken of as a geotaxis. If an organism moves toward the source of gravity it is said to be positively geotactic. If it moves away from the source of gravity it is spoken of as being negatively geotactic. Are the paramecia positively or negatively geotactic? (2) Is there any other factor which may account for the concentration of paramecia in this tube aside from gravity? (3) If your answer to Question 3 is "yes," how would you devise additional experiments to eliminate all variables except gravity? (4)

Return to observe the covered test tube placed on its side. A response to light is called a phototaxis. At which end of the tube are the paramecia concentrated? (5) Are paramecia positively or negatively phototactic? (6) Why could this experiment not have been done with the tube in a vertical position, as for geotaxis? (7) Can you suggest any value to the organism of its particular response to light? (8) Thigmotaxis involves response to contact. If time allows, devise a simple experiment to determine if an organism responds positively or negatively to contact.

Part B:
Response to Electricity

A tactic response to electricity is referred to as galvanotaxis. In a small dish, watch glass, or depression slide place enough *Paramecium* culture to cover the bottom of the container. Place the ends of the wires from the battery in the dish 1 to 2 cm apart. This distance can be increased in order to obtain a more striking response if the dish is large enough. One wire is positive; the other negative. The bare ends of the wire serve as electrodes and the current passes through the culture medium. After the current has been turned on a few minutes note the location of the paramecia. Do they respond to the electric current? (9) Are they negatively or positively galvanotactic? (10) Do they congregate at one electrode or equally at both? (11) At which electrode do they congregate, positive or negative? (12)

By means of a reversal switch or by changing the wire attachment at the battery, reverse the direction of the current. Is there a change in the direction of movement of the paramecia in response to this? (13)

Electrical phenomena are common in living cells where a temporary change in **potential** (electrical charge) frequently passes from one end of a cell to the other in response to stimulation.

Part C:
Response to Chemicals

A tactic response to chemicals is referred to as chemotaxis. The response of paramecia to food is a chemotaxis. On the basis of your observations in Exercise 19-3, is this a positive or negative chemotaxis? (14) Dip a small piece of thread into the acid solution. Place it across the middle of a drop of *Paramecium* culture on a slide and add a cover glass. Notice the reaction of the paramecia. Does the acid cause a positive or a negative chemotactic response in them? (15)

If time allows, conduct additional experiments using acids, bases, salts, or sugar, etc., to determine the response of paramecia to various chemicals.

Prepare a statement summarizing your observations on the behavior of *Paramecium*. Include the variety of environmental stimuli to which you have seen the paramecium respond and the way in which it has responded to each. Have you seen any evidence of learned behavior? (16) Is the response of paramecia to these environmental stimuli predictable? (17) Despite the lack of a nervous system, does *Paramecium* appear to have a co-ordinating mechanism of some kind? (18) Contrast the behavior of *Paramecium* with that of a dog, cat, or some other animal with which you are already familiar. (19)

REPRODUCTION

IN *PARAMECIUM*

From earlier laboratory work on microorganisms and plants you have come to realize that reproduction may be either asexual or sexual. These same reproductive patterns seen in microorganisms and in plants apply to animals as well. *Paramecium* is no exception.

■ The purpose of this exercise is to study both types of reproduction as they occur in *Paramecium*.

MATERIALS (Parts A and B)

Paramecium culture
Mating types of *Paramecium* (two cultures)
Methyl cellulose
Slide and cover glass
Compound microscope
Prepared demonstration slides of fission and conjugation in *Paramecium*
Iodine stain
Pipettes

PROCEDURE

Part A:

Fission

In the last several exercises dealing with paramecia you have undoubtedly seen animals which do not conform to the slipper-shaped outline used classically to designate *Paramecium*. In this exercise we will examine a culture of paramecia which is well fed and healthy and in which a number of forms should be seen that look as if a belt had been tightened around their middles.

Place a drop of *Paramecium* culture on a slide prepared with a ring of methyl cellulose, as in Exercise 19-1, and cover with a cover glass. Locate a paramecium which seems to be constricted in the middle. It is dividing into two animals by the process of *fission*. Perhaps you can see several stages of this process. Place on the slide a drop of iodine which will kill and stain the paramecia. By means of the stain, the nucleus in the cell should be visible. Is it similar in appearance to the nucleus of a paramecium not undergoing fission? (1) What is the general shape of the nucleus in the animals undergoing fission? (2) Carefully examine the nucleus under high power. The large structure is called a macronucleus. If possible, locate a smaller spherical structure which seems to be embedded in the surface of the macronucleus. This is the micronucleus. Add this structure to your drawing. Is there more than one micronucleus? (3)

Now change back to low power and look for several stages in the process of fission. Make several sketches beginning with what you regard as the onset of fission and carrying through until two animals appear to be held together only by a narrow band of cytoplasm. What will be the result of the completion of this constricting process? (4) Which animal is the parent? (5) What process studied previously is similar to this type of reproduction? (6) Would it be logical to say that growth of a many-celled organism by cell division is a form of reproduction? (7) Is fission a sexual or an asexual method of reproduction? (8) Why? (9)

Part B:

Conjugation

In the cultures provided, or by means of prepared slides, locate stages where two paramecia seem stuck together side by side. These animals are undergoing a reproductive process known as **conjugation**. Draw an outline of two paramecia in conjugation. How can you tell by

observation whether the paramecia are undergoing conjugation or fission? (10) Stain the paramecia undergoing conjugation as you did the ones undergoing fission. Notice the condition of the nucleus. Is it similar to the condition observed in the animals undergoing fission? (11) On the basis of your observations, what seems to be occurring between the two paramecia undergoing conjugation? (12)

Do you notice any difference between the two paramecia undergoing conjugation? (13)

In 1937 Tracy M. Sonneborn discovered that two conjugating individuals were different in a certain way, even though the differences were not structurally apparent. The conjugating individuals belong to what are called different **mating types**. Thus, in effect, they correspond to males and females. An individual of mating type I will conjugate with an individual of mating type II. They will not, however, conjugate with other mating type I individuals nor will type II mating types conjugate with one another. On the basis of the existence of mating types, would you describe conjuga-

tion as an asexual or sexual process? (14) Why? (15) Do you think fission or conjugation would be the most valuable type of reproduction for the continuation of the species under changing environmental conditions? (16) Why? (17)

In Exercise 19-1 you formulated some hypotheses regarding structure and possible function. On the basis of the work in the subsequent exercises, relabel the *Paramecium* drawing (Figure 19-6-1) putting in what you now believe to be the correct functions of the various parts. Label the parts with which you have become familiar.

On the basis of this series of exercises, formulate a statement indicating how you would go about finding out the function of any particular structure. Could you also then find out in which structure a particular function was localized? (18)

On the basis of your work with *Paramecium*, do you think it is a simple or a complex animal? (19) Why? (20) How does *Paramecium* serve as a good experimental animal when studying structure and function? (21)
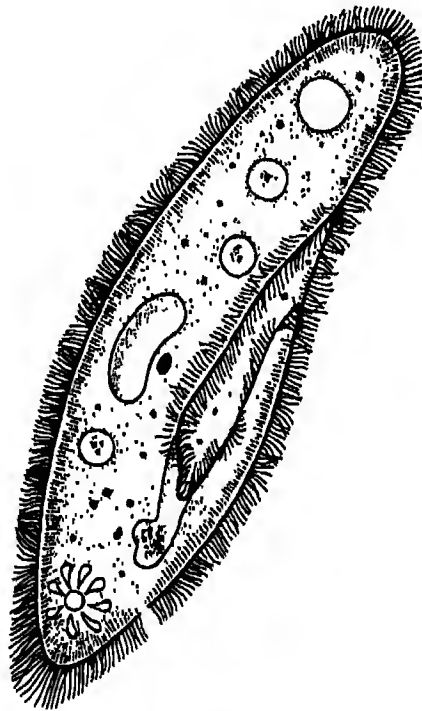
Figure 19-6-1

PARASITIC AND FREE-LIVING

WAYS OF LIFE

The term *worm* is used commonly to describe any long, slender, soft-bodied animal—such as earthworms, tapeworms, maggots, adult forms of some insects, and even certain legless salamanders. Wormlike animals, therefore, occur in a great number of animal phyla and you must be specific as to what you mean when you use the term *worm*. In this exercise we will deal with two different kinds of worm. One will be a **parasite** (living at the expense of another organism), a roundworm belonging to the phylum Nematoda. The other will be a free-living (nonparasitic) worm, the earthworm, belonging to the phylum Annelida.

■ The purpose of this exercise is to study the differences between worms adapted to a parasitic existence and those adapted to a free-living existence.

MATERIALS (Parts A and B)

Live vinegar eel culture
Live earthworms
Preserved earthworms
Preserved *Ascaris*
Prepared slides of cross sections of *Ascaris* and earthworm
Hand lens
Stereoscopic dissecting microscope
Compound microscope
Straight pins
Pinning tray
Dissecting needle
Razor blade
Paper toweling
Container of loose, moist, soil
Slide and cover glass
Medicine-dropper pipette
Forceps

PROCEDURE

Part A:

Vinegar Eels and Ascaris

By means of a medicine-dropper pipette place a drop of solution from the bottom of the vinegar eel culture on a clean glass slide. Cover and observe it under low and high power with the compound microscope. The small, wormlike animals are vinegar eels. Are they slow-moving or rapid-moving? (1) Describe the type of motion shown by these small worms. (2) Does this seem to be an efficient way of locomotion? (3) Do these animals live in a medium of high or low pH? (4) Do you suppose they would do equally well in a medium of neutral pH? (5) How could you easily test for the answer to this question? (6)

The type of locomotion demonstrated by these free-living vinegar eels is closely correlated with the pattern of their body muscles. Which direction would the muscles have to run in the body of the worm in order to produce the kind of motion produced when muscles can only contract? (7)

A larger member of the phylum Nematoda is *Ascaris*, a worm parasitic in the intestines of pigs and other vertebrate animals. Look carefully at several ascarid worms on display. Do you notice any differences between them? (8) Do you see any evidence of external sense organs? (9) Do you see any evidence of cilia or other types of structures for movement? (10) Stroke the surface of the ascarid worm. Is it smooth or rough or provided with projections? Describe it. (11) By means of a hand lens or stereoscopic microscope locate any body openings. Is there a definite head to *Ascaris*? (12)

If time allows, perform the following dissection. If not, your teacher will provide a demonstration. Put a pin through each end of the ascarid worm and pin it to a wax dissecting

pan. Using a sharp razor blade make a shallow, longitudinal incision, being careful to cut only through the body wall. Start at the anterior (front) end and cut along the dorsal (back) surface, exposing the body cavity and the digestive tube. Use pins to hold the cut edges apart and, upon completion of the incision, add water to the tray to hold up the structures so they can be more easily seen. The digestive tract is a single tube running from mouth to anus.

Notice the white, threadlike reproductive system. By means of a needle, uncoil it and notice that all parts form a continuous structure that finally opens to the outside. Comparing reproductive systems of the *Ascaris* dissected by other students, are all the reproductive systems the same? (13) Does this suggest that these animals are divided into males and females? (14) Do the reproductive tracts all open to the outside at the same spot? (15) Compare the differences observed in the reproductive system with the differences in external appearance of the animals. Does a certain external appearance always go with a certain type of reproductive tract? (16)

Remembering that *Ascaris* lives in the intestines of vertebrate animals, form a series of hypotheses regarding what you believe its respiratory system, digestive system, outer covering, nervous system, and muscular system would necessarily be like. (17)

Part B:
The Earthworm

Place a live earthworm on a piece of damp paper toweling. In what two ways is it obviously externally different from *Ascaris*? (18) Contrast the type of locomotion in the earthworm with that seen in the vinegar eel. (19)

Examine the earthworm closely with hand lens or stereoscopic dissecting microscope. On the basis of your observations of openings to the interior, would you expect that this animal has a complete digestive tube? (20) Does the earthworm have distinct anterior and posterior ends? (21) Does the common direction of movement tell you anything about which is the mouth end of the animal? (22) In the mid-dorsal (middle of the back) area a dark-colored blood vessel can be seen clearly through the skin. This is the dorsal blood vessel. Notice the waves of contraction moving the blood along in this vessel that behaves like a heart. In which direction does the blood flow, an-

teriorly or posteriorly? (23) Choose a definite spot on the dorsum of the worm and count and record the number of contractions per minute. (24) Count your own pulse rate and compare it with the contractions of the earthworm. Which is faster? (25) How can you account for this difference in rate of contraction? (26)

Are sense organs apparent on the surface of the earthworm? (27) Touch a live earthworm with the point of a pencil near the head and at other places and observe the reactions. (28) Shade parts of the towel with your hand or a dark piece of paper. Does the earthworm move toward light or away from it? (29) Observe earthworms placed on the top of loose, moist soil. What is their reaction? (30) Watch the earthworm digging in the soil. Can you make any deductions as to how it burrows through the earth? (31)

If time allows, you may be able to dissect earthworms individually, as follows. If not, your teacher will show you a dissected earthworm.

Pin a preserved earthworm at both ends to the wax bottom of the tray. With a razor blade, make a shallow, longitudinal incision slightly to one side of the midline starting at the enlarged ring (clitellum) about thirty segments from the anterior end. Extend this shallow slit all the way to the anterior tip of the worm through the dorsal body wall, being careful not to cut too deeply.

With forceps and razor blade, loosen the body wall and pin it back against the bottom of the tray to expose the internal organs. Be careful not to tear the surface layers. What is one feature that makes the dissection of the earthworm more difficult than the dissection of *Ascaris*? (32) Is there any relation between the crosswise partitions on the interior of the worm and the rings on the outside of the worm? (33) The earthworm is divided into segments or rings (annuli). Does the term in parenthesis suggest to you where the phylum Annelida gets its name? (34) Is the digestive tract of the earthworm simpler or more complex than that of *Ascaris*? (35) How does the type of digestive tract seen in each of these animals correlate with their respective ways of life? (36)

On the preserved specimen locate the dorsal blood vessel seen earlier on the living specimen. In the anterior region note the connecting vessels joining the upper dorsal blood vessel with the lower ventral blood vessel. In view of your observation of the dorsal blood vessel, which way will the blood be flowing through

these connectives and in the ventral blood vessel? (37)

Just to the side of (lateral to) the circulatory connectives, there are some fairly large, white, reproductive organs of the worm. How do these compare in location, size, and shape with those of *Ascaris*? (38) In earthworms are males and females separate individuals? (39)

If time allows, observe cross sections of *Ascaris* and earthworm under the microscope to compare structures in both. How do these cross sections compare? (40) Is there an advantage to having the digestive tract in a hollow space within the body? (41) In the earthworm cross section you can see the dorsal and ventral blood vessels and a solid structure which is the nerve cord. Is this dorsal or ventral? (42)

Notice that the digestive tube is not circular in the earthworm, but has an indentation at its top. Of what possible value can this be to the digestive system of the earthworm? (43) Can the different ways of life of *Ascaris* and the earthworm explain the differences in their digestive tracts? (44)

In the cross section of the earthworm, sections of the tubes of the excretory system may be seen and perhaps even their openings to the outside observed. In either cross sections or entire dissected earthworms have you seen any evidence of a respiratory system? (45)

Prepare a concluding statement contrasting the structures and functions of an earthworm with those of *Ascaris* and relating these to the different ways of life of these two worms. (46)

ANIMALS WITH

JOINTED APPENDAGES

In previous laboratory exercises the animals studied have had no jointed appendages. In this exercise we will deal with two animals, the grasshopper and the crayfish, each representing a different way of life within the phylum Arthropoda (jointed-foot animals). Since more than 75% of all the species of animals on earth today are arthropods, they constitute an important phylum indeed.

■ The purpose of this exercise is to learn something of the structure and behavior that characterize arthropods and to correlate and contrast the structure of the arthropod body with that of previously studied animals.

MATERIALS (Parts A and B)

Living grasshoppers (immature and adult)
Preserved adult grasshoppers and immature grasshoppers
Live and preserved crayfish or lobster
Covered dish or plastic box to contain live grasshopper
Dish with clear pond water for live crayfish
India ink
Stereoscopic dissecting microscope or hand lens
Small, freshly killed fish
Leaves or grass for feeding grasshopper

PROCEDURE

Part A:

The Crayfish

Observe a living crayfish (or lobster) in several inches of water in a dish or in an aquarium and study Figure 20-2-1. By touching the antennae (feelers) with a pencil, you will cause the animal to make an extreme avoidance reaction. In what direction does it move to get away from this stimulus? (1) How does it propel itself rapidly in this direction? (2) When undisturbed on the bottom of the dish, in what direction does the crayfish move? (3) By what means does it move more slowly along the bottom? (4)

Tap the crayfish with a pencil. Is its outer covering soft or hard? (5) Does its outer covering suggest a greater or lesser degree of protection than was observed in the earthworm or *Ascaris*? (6) Can the crayfish move in the accordionlike fashion seen in the earthworm? (7) At what points can the crayfish move its body? (8)

The covering of the crayfish is called an exoskeleton. It has no internal bony skeleton like that of a human being. On the basis of your observations what would you consider to be an advantage of an exoskeleton? (9) A disadvantage? (10) Does the crayfish exoskeleton show evidences of segmentation similar to those seen in the earthworm? (11) Looking at the exoskeleton, can you see evidences of regular segmentation from the head to the tail? (12) The exoskeleton is something like a suit of armor and does not grow. If this is the case, how can the crayfish get bigger and bigger? (13)

Remember, it is the jointed appendages that give this phylum its name. How many pairs of appendages can you count on the crayfish? (14) Are all of the appendages identical? (15) Do you see evidences of specialization in the appendages? (16)

To the dish of clear water containing the crayfish add a drop of ink immediately posterior to the cephalothorax. What does the movement of ink tell you about the activity of some of the appendages even when the crayfish is apparently at rest? (17) Which appendages
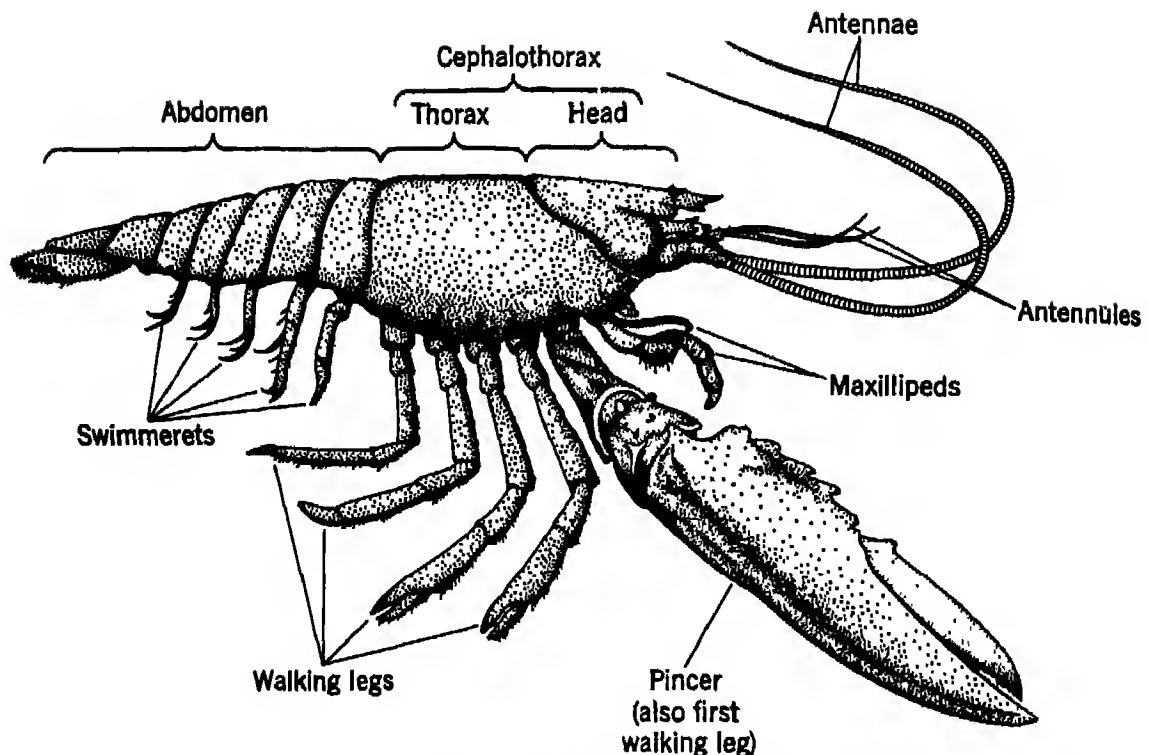
Figure 20-2-1 The lobster above is also a crustacean. The crayfish is so similar to the lobster that this drawing may be used for studying either.

ages are responsible for the movement of the ink? (18)

Put a small, freshly killed fish in the dish with the crayfish. If the animal is sufficiently hungry you may see it capture and eat the fish. Pay particular attention to the use of its appendages during this process.

Turn now to a preserved specimen of the crayfish. Lift the lateral part of the exoskeleton covering the area over the walking legs of the crayfish. This area is called the cephalothorax, for it covers both the head and the thorax. The feathery structures seen along the side are gills. To what are they attached? (19) What connection does the location of the gills have to the current that was set up by the appendages when the ink was added to the water? (20) With gills as its respiratory structures, could the crayfish be a successful land animal? (21)

What specialized sense organs do you notice on the crayfish? (22) Touch various parts of the live crayfish to see which body areas are most sensitive to touch. At which end of the body are the sense organs located? (23)

Compare the crayfish to the earthworm in respect to (a) locomotion, (b) respiration, (c) sense organs. Remember, that as arthropods are more closely related to annelids than to any other phyla, you would expect many similarities between the two.

Part B:
The Grasshopper

Examine a living grasshopper and refer to Figure 20-2-2. In what ways is it similar to the crayfish? (24) In what ways is it dissimilar? (25) Two different patterns of locomotion were observed in the crayfish. How many can you observe in the grasshopper? (26) What appendages are obvious in the grasshopper that were not seen in the crayfish? (27)

Does the grasshopper have an exoskeleton? (28) Notice the demonstration of living young grasshoppers. In what ways are they different from the adults, other than being smaller in size? (29) What changes occur as the grasshoppers become larger? (30) When a grasshopper hatches from the egg it looks like a tiny adult with the exceptions you were just asked to notice. What must occur each time it is to grow larger? (31) In your textbook there is a discussion of the development of the fly (in the section on Redi's experiments). How does the development of the fly, as described in the textbook, differ from the development of the grasshopper, as you see it here? (32) What stages in the life history of the fly do not seem to occur in the life history of the grasshopper? (33) The series of changes which may occur in the form of insects and other animals from egg
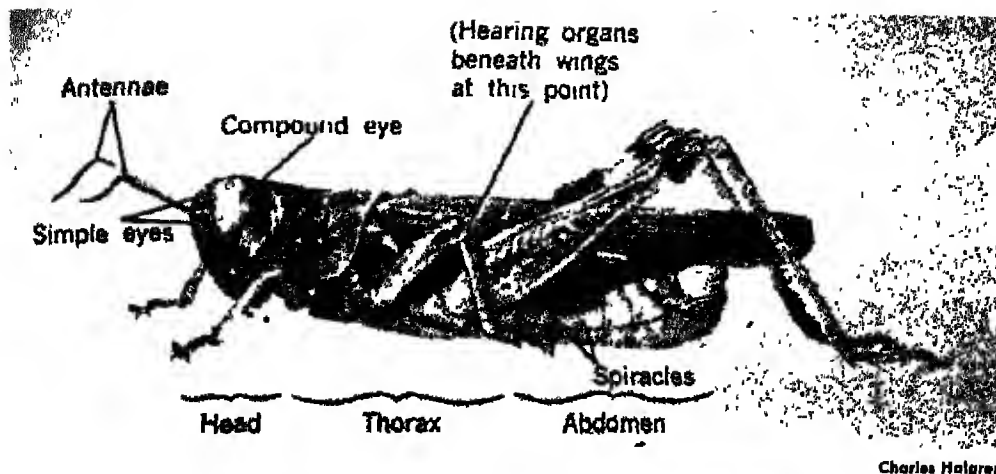
Figure 20-2-2 External structure of the grasshopper.

to adult is called metamorphosis. In the fly, metamorphosis is said to be complete. In the grasshopper it is said to be incomplete. On the basis of your preceding observations, why is this necessarily so? (34)

If the grasshopper is hungry it may be induced to eat leaves or grass. If so, notice carefully its eating habits and contrast them with those seen in the crayfish.

Compare the sense organs seen on the grasshopper with those on the crayfish. In what ways are they similar? (35) In what ways different? (36)

Do you notice any gills on the grasshopper? (37) Assuming that it has a respiratory system, what would you look for on the exoskeleton to indicate its presence? (38)

From your observations of the behavior of the grasshopper, for what kind of environment would it seem best fitted? (39)

Do you see any sexual **dimorphism** (difference between males and females) in the grasshopper? (40) Are separate sexes present in the grasshopper population? (41) Did you notice any sexual dimorphism in the crayfish? (42) Sexual dimorphism is not always associated with separate sexes.

Based on your observations of the invertebrates — *Paramecium*, *Ascaris*, earthworm, crayfish, and grasshopper studied thus far — describe changes in structure and behavior noted from protozoan to arthropod. How can you account for the changes you have noticed? (43)

FORM AND FUNCTION

IN THE FROG

This exercise is concerned with the internal structure of the frog and the functions of some of its parts.

We will begin the exercise with a freshly killed frog. The frog has been pithed, a process whereby the brain and spinal cord are severed at the connection between the vertebral column and the skull. This is a simple, painless process when correctly done, and the frog is effectively killed at the moment of pithing even though certain body processes may continue for some time.

Remembering the discussions in the early chapters of the textbook concerning definitions of life and death, the pithed frog poses an interesting problem of time of death. Various processes continue for varying lengths of time after pithing, depending upon temperature, humidity, and other environmental factors. If a pithed frog is placed in a refrigerator it may be possible to observe the heart still beating after a day, and you can stimulate muscle twitching by nerve stimulation several days after storage. Remember, however, that insofar as continuance of life is concerned the frog is as effectively dead at the moment of pithing as if its heart had been completely removed or as if its head had been chopped off. After a procedure such as pithing, how does the continuation of the processes that we commonly associate with life alter your definition of death? (1) Does the fact that the animal does not appear to die all at once alter your understanding of what life is? (2)

■ The purpose of this exercise is to observe the internal structure and function of some of the major organs in the body of the frog. These organs and their functions are essentially the same in all chordates. The study of the organs in the frog's body cavity will contribute to your understanding of human physiology and anatomy.

MATERIALS

Pithed frog

Dissection instruments, scissors, scalpel, forceps, needles

Dissecting pan

Pins

Pipette

Watch

Hand lens or stereoscopic dissecting microscope

Caffeine or adrenaline hydrochloride mixture

Saturated salt solution

Distilled water

Culture dish or Petri dish

10% acetic acid

50% alcohol or 5% formalin as preservative

Centimeter ruler

First Period. Fasten the pithed frog, ventral (belly) side up, to the bottom of the dissecting pan with pins placed through the ends of the four limbs. Raise the skin from the mid-ventral surface with forceps and make a longitudinal (antero-posterior) incision along the midline from the pelvis (the region of the body between the base of the hind limbs) to the tip of the lower jaw. Free the skin from the area between the forelimbs, and make short lateral (sidewise) cuts in the skin at the forelimb region and at the posterior end of your longitudinal incision to form flaps which can be pinned out at the sides. Is the skin of the frog closely bound to underlying muscles? (3) Lift the abdominal muscles and make a longitudinal cut in the body wall as before. Make this incision, however, a little to one side of the midline in order not to cut the prominent abdominal vein which is visible as

Heart

Lung

Liver

Ovary (filled
with eggs)

Pancreas

Mesentery

Stomach

Small intestine
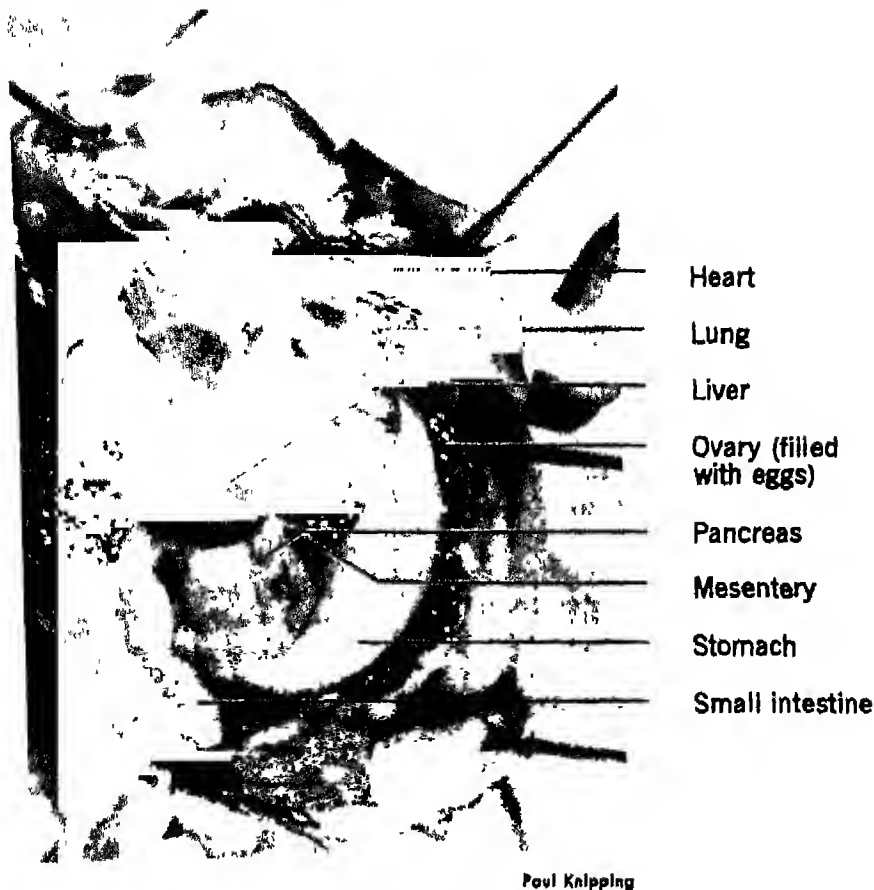
Figure 20-3-1   Internal structure of the frog.

a dark line down the center of the abdomen. Cut anteriorly through the sternum (the skeleton between the forelimbs) and remove about 1.5 cm of it. Be careful not to injure the organs beneath the sternum. Pull the forelimbs a little farther apart and fasten them again with pins. Pin back the abdominal muscles after making lateral incisions, as you did for the skin. Figure 20-3-1 locates some of the internal structures. Notice carefully the way the organs lay in the body cavity. Locate the heart, which lies anteriorly in the body cavity. Does its beating indicate that the frog is still alive? (4) Count the number of heartbeats per minute. (5) Is the frog heartbeat faster or slower than yours? (6) Carefully slit the thin, transparent membrane surrounding the heart. How many chambers are apparent in the frog's heart? (7) The anterior chambers are the atria. The posterior, the ventricle. Do both of the atria contract at the same time? (8) Does the ventricle contract at the same time as the atria? (9) In what direction does the heartbeat travel—from the atria to the ventricle or from the ventricle to the atria? (10) Locate the large artery leaving the ventricle and try to trace its major branches. Can you determine the organs to which this

large artery goes? What type of blood would you expect to leave the heart and go to the lungs? (11) Would this blood be traveling in an artery or a vein? (12) What type of blood would you expect to see in the vessels going to the head region? (13) Would this blood be carried to the head in an artery or in a vein? (14)

By means of a pipette put a drop or two of cold water on the heart. Do you notice any difference in rate of heartbeat? (15) After a minute or two, repeat with warm water. Is there any difference in the heartbeat now? (16) Add a drop or two of caffeine solution or adrenaline hydrochloride to the heart. How does this affect the rate of heartbeat? (17) Substances which slow things down are said to be inhibitors. Substances which speed up reactions are accelerators. How would you categorize cold water, warm water, and caffeine or adrenaline? (18) Insert a pipette into the glottis, which is a short slit on the floor of the oral cavity. Gently inflate the lungs by blowing carefully. The lungs burst very easily. Note the increase in size and the texture of the wall of the lung. What feature of the frog lung makes passage of oxygen and carbon dioxide more rapid? (19) Are the lungs large or small in relation to the

body cavity size? (20) Is there a separation of any kind between the lungs and the other organs of the body cavity? (21)

Expose the whitish stomach and large intestines and small intestines in the posterior part of the body cavity. Place a few drops of concentrated salt solution along the surface of the intestine. Is any reaction noticeable after a minute or two? (22) In what direction do peristaltic waves of contraction move? (23) What is the funtion of these peristaltic waves in the process of digestion? (24)

Trace the small intestine from the stomach to the posterior large intestine. Cut out a piece about 1 cm long at a distance of about 1.5 cm from the stomach and the same distance from the large intestine. Drop the freed piece of intestine into a dish of distilled water and watch the reaction. Describe the reaction of the intestine in the distilled water. (25) The distilled water is said to wash the calcium out of the cells of the intestinal wall. If this is true, what hypothesis can you make concerning the function of calcium in relation to muscle action? (26) Unpin the frog, close the flaps, and tie a string around the middle of the animal to keep the flaps closed to protect the internal organs. The frogs may be wrapped in damp toweling and stored in a refrigerator if they are to be used tomorrow. However, if there is a longer interval between laboratory classes, the frogs should be placed in a 50% alcohol solution or 5% formalin for future use.

Second Period. After you remove your frog from the refrigerator, you may be surprised to find the heart still beating. If a 10% acetic acid solution is dropped on the skin, you may still get a withdrawal reaction. If one of the muscles of the leg is pinched it may move. What does this tell you about the viability of parts of an animal? (27) If your frog has been stored in either formalin or alcohol, carefully wash it in running water and pin the specimen in the dissecting pan as in the first period. Today's exercise will be primarily concerned with locating other organs of the body and attempting to correlate them with the proper function. Follow the directions carefully and make sure you have located each part before you proceed further.

The heart, which you located in the first laboratory period, will be used as a point of departure. Just posterior to it are the rather large lobes of the liver. These are a reddish-brown color. How many lobes are there to a frog's liver? (28)

In all dissection directions, the terms right and left are used. This terminology always refers to the right and left of the animal being dissected. Can you see possible difficulties if the right or left of the person doing the dissection were used? (29) In the lobe of the liver to the right, there is a small, greenish sac, the gall bladder. With the aid of a hand lens or stereoscopic dissecting microscope, locate the bile duct, which appears as a thin, light-colored tube leading from the gall bladder to the duodenum, the part of the small intestine just next to the stomach. Under the lobe of the liver to the left is the whitish stomach. In many cases it is only slightly larger in diameter than the small intestine. Its lower end has a constricted area, the pylorus, which controls the opening of the stomach into the intestine. Why should there be a valve at the union of stomach and intestine? (30) When food passes from the stomach into the intestine, the secretions of the gall bladder and those from the pancreas are added. The pancreas is a flat, narrow structure, usually pinkish in color, lying between the inner surface of the stomach and the duodenum.

You have already removed most of the small intestine in the previous laboratory period. You have already located the large intestine. When you cut away the section of the small intestine in the previous laboratory period, how was it attached to the body wall? (31) Is there a similar membrane holding the stomach and the large intestine in place? (32) The supporting membrane which holds the digestive tract and the other organs which lie in the body cavity is called the mesentery. Attached to this mesentery, just anterior to the large intestine, and slightly dorsal to it, is a small, reddish, pea-shaped organ, the spleen. Look for attachments of the spleen to the digestive tract. Do you find any tubes or ducts which would indicate that the spleen is part of the digestive system? (33) Return now to the large intestine, which is a relatively short, pear-shaped structure opening to the exterior through the anus. Why is it called the large intestine if it is shorter than the small intestine? (34) Remembering the size of the piece of intestine cut out in the last laboratory period, how many centimeters of small intestine do you find in this frog? (35) How many centimeters of large intestine? (36) Compare the diameters of the large and small intestine. How many millimeters across is the small intestine? (37) How many millimeters across is the large intestine? (38) The intestine opens into a chamber called the cloaca. This is a com-

mon chamber into which the digestive system, reproductive system, and excretory system open. Is there a cloaca in the human? (39)

If your frog is a female, the ovary is probably full of dark-colored, mature eggs. Carefully remove the ovary and the eggs from one side of the body cavity. The oviducts are white, coiled tubes that are attached to the dorsal body wall on each side of the midline. Under the oviduct and attached by a mesentery to the dorsal wall you will find a flat, reddish, oval organ, the kidney. There is one on each side of the body. Attached to the anterior end of the ovary will be a cluster of yellowish, fingerlike structures, the fat bodies. If the eggs are not well developed, the oviducts will be quite small in size and very tightly coiled. The posterior end of the oviduct is called the uterus (plural: uteri). The uteri open into the cloaca. The ovary will be held in place by a mesentery attached along the edge of the kidney. It is irregular in shape and under the hand lens will show very small eggs developing within it. This gives it a granular appearance.

If your frog is a male, to the surface of each kidney will be attached (by a mesentery) a small, light-colored, bean-shaped body—the testis. In the leopard frog from some parts of the United States, a very small coiled tube borders the outer edge of the kidney and extends posteriorly to enter the cloaca. This tube is an oviduct which is nonfunctional. It is spoken of as a vestigial oviduct and is not present in bullfrogs. It has nothing to do with the passage of the sperms to the outside. The sperms enter the kidneys and pass by way of the ureters to the cloaca. What does the presence of the vestigial oviduct in the male indicate about the reproductive system of the frog? (40)

Along the ventral surface of the kidney will be seen a light band of yellowish material embedded in the kidney tissue. This is the adrenal gland. With a hand lens locate the very thin tube that leads from the posterior end of each kidney to the cloaca. This is the ureter. Finally, find the thin-walled urinary bladder, which is a two-lobed sac emptying into the cloaca.

On the basis of your observations above, trace the route of an egg from the ovary to the cloaca by listing the structures, in order, through which it must pass. (41) Repeat this path for the sperm from the testis to the cloaca. (42) Trace a drop of urine from the kidney to the outside in this same manner. (43) Pay particularly close attention to the relationship of the bladder and the ureter to the cloaca.

Return now to the stomach for further observation. With a scalpel or fine-pointed scissors, slit open the stomach along its full length at its outer curvature. Spread it apart and observe the internal surface. Is food present inside the stomach? (44) If so, what kind of food? (45) Is the frog a carnivore or an herbivore? (46) Describe the internal surface of the stomach. Is it smooth or rough? (47)

Slit open the portion of the small intestine remaining attached to the stomach. Observe its internal structure under a hand lens or stereoscopic dissecting microscope. Is it smooth? (48) Is it similar to the inside of the stomach? (49) What possible adaptation for digestion does the inner lining of the small intestine offer? (50)

If time allows, it may be possible to observe parts of the muscular and nervous systems and the circulatory system in greater detail.

Write a brief paragraph indicating why study of the internal organs of the frog and their functions would be of value to you in understanding the organs and functions of the human body. (51)

ANIMAL CLASSIFICATION

Your textbook traces the development of "naming" from the time of John Ray, who first named species, through Linnaeus, who developed the modern binomial system of classification. In our previous laboratory exercises on animals we have used representatives of different animal phyla and have given them only common names.

For work in biology it is essential to have the proper scientific name of the animal or plant with which you are working so that the experiment can be repeated by another scientist, perhaps at a distance, using essentially the same material. It would not, therefore, be sufficient to say simply that one worked with a crayfish. There are over 200 species and subspecies of crayfish in the United States alone. In order to make sure that the work is truly repeated, it is necessary to specify what particular species of crayfish should be employed. Therefore, precise naming of animals is invaluable in all branches of biology. In the earlier days of naming animals the science was called taxonomy, which meant merely the assigning of names. Nowadays we speak of it as systematics, involving not only the assigning of names but also the arrangement of animals in a logical systematic order that shows their relationship to one another.

■ The purpose of this exercise is to familiarize you with methods of classifying animals and of identifying animals whose names are already in existence. In this study we should also become familiar with the great diversity within the animal kingdom and come to appreciate some of the relationships between the various groups of animals.

PROCEDURE

Animal classification relies principally on external morphology. Review in your textbook the relationship of the term homology, implying evolutionary similarity and a common embryonic origin, and the term analogy, which implies superficial resemblance. In classification the greater the number of homologous structures two animals have in common, the more closely they are regarded as related.

In this exercise, while we will not attempt to give names to animals, we will attempt to find names that have already been given.

The simplest method of locating the name of an unknown animal is by the use of an appropriate key. A key is literally a structural map of a phylum, class, genus, species, or other taxonomic unit. It operates by successively eliminating choices until the correct choice is reached. In this exercise the keys will be extremely simple and will be based only on readily visible external characteristics, wherever possible. It is obvious that the more detailed a key is, the more accurate it is and the smaller the systematic unit will be in which the key will place an animal. Therefore, in dealing with the following simple keys no provision is made for occasional exceptions to the rule. Keys are available in a great number of books for practically every existing group of plants and animals. However, as the use of a specialized key involves a great deal of specialized terminology, our keys will be necessarily simple. You will find that the simpler the key the greater the chances of error, for one word or a brief statement will not be sufficient for identifying all the animals in a given phylum, class or order.

MATERIALS

Preserved or mounted specimens of ten major animal phyla

Preserved or mounted specimens of four major arthropod classes

Preserved or mounted specimens of seven chordate classes

Additional mounted and preserved specimens as desired and available

The easiest kind of key to imagine is one which just pairs short phrases or preferably two single words. The following key for ten animal phyla is made as simply as possible.

Using the representative animals available, identify the phylum to which each belongs. Remember that in each couplet of the key you will find either that the animal belongs at that point or that you go on to the next number until it is properly described.

Key A—Simple Key

1. Microscopic. . . . Protozoa
1A. Visible to the unaided eye. . . . go on to No. 2

2. Holes. . . . Porifera
2A. No holes. . . . go on to No. 3

3. Body a two-layered sac with one opening. . . . Coelenterata
3A. Body not saclike or with two openings, three layers or more. . . . go on to No. 4

4. One opening to the digestive tract. . . . Platyhelminthes
4A. Two openings to the digestive tract. . . . go on to No. 5

5. Radially symmetrical. . . . Echinodermata
5A. Bilaterally symmetrical. . . . go on to No. 6

6. Nonsegmented worm. . . . Nematoda
6A. Segmented or with shell. . . . go on to No. 7

7. With a shell. . . . Mollusca
7A. Segmented. . . . go on to No. 8

8. Legless. . . . Annelida
8A. With appendages. . . . go on to No. 9

9. Exoskeleton. . . . Arthropoda
9A. Endoskeleton. . . . Chordata

The more you know about animals and their structures the more detailed a key you can use. The next key is exactly like the one above except that it lists a few more characteristics. It will allow you to identify the forms more accurately because it takes into account a few more exceptions. Using the same animals as for Key A, re-key them with Key B. Are the results the same? How do you account for any differences? (1)

Key B—More Detailed Key

1. Usually microscopic in size; body of a single cell; if of more than one cell each cell almost independent of the others. . . . Phylum Protozoa

1A. Usually visible to the naked eye; multicellular; cells dependent on one another. . . . go to No. 2

2. Body wall covered with holes; radially symmetrical or asymmetrical; adults fastened to one spot. . . . Porifera
2A. Body wall lacking numerous openings; radially or bilaterally symmetrical; sessile or free-living. . . . go to No. 3

3. Saclike body of two cell layers; radially symmetrical; digestive cavity with one opening; tentacles frequently present. . . . Coelenterata
3A. Body with three embryonic cell layers; bilaterally symmetrical, at least in larvae. . . . go to No. 4

4. Digestive cavity with one opening; body flattened; mostly parasitic. . . . Platyhelminthes
4A. Digestive cavity with two openings; body more rounded. . . . go to No. 5

5. Adults radially symmetrical; body pattern in "five's"; all marine. . . . Echinodermata
5A. Adults bilaterally symmetrical; marine, freshwater or terrestrial. . . . go to No. 6

6. Wormlike; no shell, scales or prominent sense organs; mostly parasitic; nonsegmented. . . . Nematoda
6A. If wormlike, with shell, scales, segmentation or prominent sense organs; mostly free-living and nonwormlike. . . . go to No. 7

7. Nonsegmented with shell; muscular foot; mostly slow-moving or sessile. . . . Mollusca
7A. Segmented, mostly with no shell or muscular foot; mostly rapidly moving and free-living. . . . go to No. 8

8. Legless worms; no prominent skeleton; segmentation obvious. . . . Annelida
8A. Mostly with jointed appendages; obvious exoskeleton or endoskeleton; segmentation frequently not obvious externally. . . . go to No. 9

9. Exoskeleton of chitin. . . . Arthropoda
9A. Endoskeleton of cartilage or bone. . . . Chordata

Keys are used not only to identify animals as to phyla but exist, as pointed out above, for all systematic categories of animals.

The following key will identify most members of four classes of arthropods. Using the

specimens available to you place the selected arthropods in the class to which they belong.

Key C—Key to Classes of Arthropods

1. Wormlike adults with many body segments and with one or two pairs of legs per segment. . . . **Myriapoda**
1A. Nonwormlike adults; fewer body segments, and with either one pair of legs per segment or none. . . . See No. 2

2. Two pairs of antennae; marine or aquatic; gills. . . . **Crustacea**
2A. One pair of antennae or none; terrestrial or freshwater; tracheal tubes. . . . See No. 3

3. Four pairs of legs. . **Arachnida**
3A. Three pairs of legs. . . . **Insecta**

Turning to the prominent classes of the phylum **Chordata**—to which we belong—the following key may serve to help you identify chordate organisms to the class in which they belong.

Key D—Simple Key to the Classes of Chordates

1. Fins or no appendages; marine or aquatic. . . . fishes. . . . See No. 2
1A. Legs, wings, or flippers, but not fins as appendages; mostly terrestrial. . . . See No. 3

2. Slimy, scaleless skin; no paired appendages and reduced fins; no jaws; snakelike. . . . **Cyclostomata**

2A. Leathery skin with small scales; prominent fins; jaws; no bones. . . . **Chondrichthyes**
2B. Scaly skin with prominent scales; jaws; bone present. . . . **Osteichthyes**

3. No scales, feathers, or hair. . . . **Amphibia**
3A. Scales, feathers, or hair. . . . See No. 4

4. Scales. . . . **Reptilia**
4A. Feathers. . . . **Aves (Birds)**
4B. Hair. . . . **Mammalia**

After you have had some experience in keying out already-known animals, use the following information to construct a key to the classes of mollusks. The five classes of mollusks are characterized in the following way:

Class Amphineura. Lacks a well-developed head and special sense organs; has gills in mantle; a dorsal skeleton composed of several separate plates (Figure 20-4-1A).

Class Scaphopoda. Has a single, tusklike shell which is uncoiled and open at both ends. Small tentacles present. Foot and mantle cavity present (Figure 20-4-1B).

Class Pelecypoda. Bilaterally symmetrical, with a shell of two halves (or valves). Many are sessile. Feed by filtering microorganisms from the water, use gills as respiratory structures. Mostly marine, but some freshwater, none terrestrial. A few swim fairly well. May reach 1.8 m (6 ft) in diameter (Figure 20-4-1C).

Class Gastropoda. Has a single shell with one opening. The shell commonly twisted in
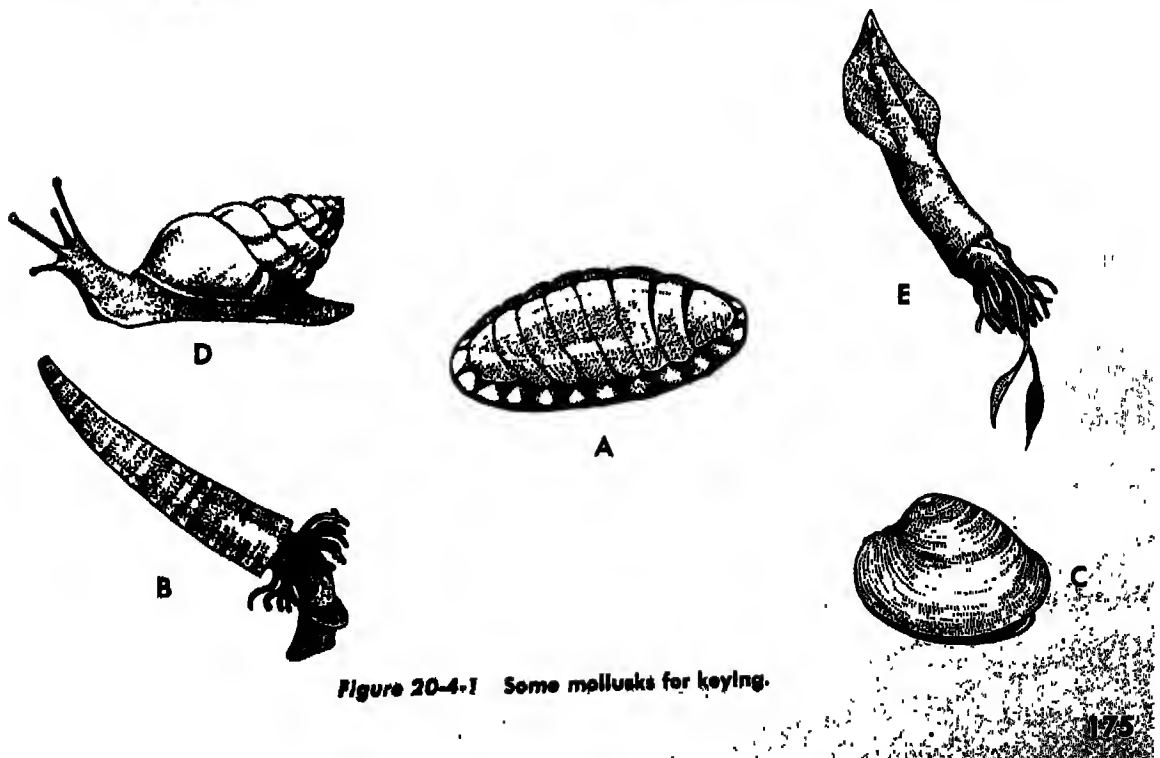
Figure 20-4-1 Some mollusks for keying.

a spiral. Marine or terrestrial. Breathing by gills or lungs. Some lack shells; their internal organs, however, always twisted as if in a twisted shell. Well-developed head and sense organs (Figure 20-4-1D).

Class Cephalopoda. Free-living, fast-moving forms, with large image-forming eyes, large and obvious tentacles, mostly with greatly reduced shells. Frequently move by means of "jet propulsion" when water is forcibly squirted from the mantle cavity (Figure 20-4-1E).

After you have constructed a key using appropriate parts of the above data, try it out with known members of each mollusk class and check it for accuracy.

Do all the classes within a phylum bear the same characteristics as the phylum? (2)

Are classes within a phylum more similar to one another than they are to classes belonging to another phylum? (3) Why? (4) What features seem to be most commonly used, as shown in this exercise, for classifying animals? (5)

If you were to dissect the animals concerned, do you think a suitable key might be made for the internal structures as well? (6)

What would be one of the characteristics of a key to the animal phyla which was absolutely foolproof and contained references to all exceptions? (7)

Would you expect a key to the orders of insects to separate insects on the basis of presence or absence of an exoskeleton? (8) On the basis of numbers of legs? (9) On the basis of presence or absence of wings? (10) On the basis of differences in body form? (11)

Do you suppose it would be possible to construct a key which would identify species of animals on the basis of their habits? (12) On the basis of their geographical distribution? (13) Their food habits? (14) Their economic importance? (15)

What would be wrong with classifying animals simply on the basis of color? (16) Butterflies, birds, and bats all have wings. Why would you not classify them together? (17)

Cats, seals, and bears are classified in the same order of mammals (Carnivora) despite the fact that superficially they bear no close resemblance to one another. Why then are they considered to be members of the same order? (18)

DIGESTION OF FOODSTUFFS

In the textbook Figure 21-7, the areas of the human digestive tract are shown. In the following table, Human Digestion, the types of foods processed in each area of the digestive tract are given.

HUMAN DIGESTION

Areas	Glands	Foods Affected	End Products
Mouth	Salivary	Starch	Maltose
Stomach	Gastric	Protein	Proteoses and peptones
Intestine	Pancreatic and Intestinal	Fats	Fatty acids and glycerol
		Starch	Maltose
		Protein, proteoses, peptones	Amino acids
		Maltose, milk sugar, table sugar	Glucose and other simple sugars

On the basis of the drawing of the digestive tract and the table it can be seen that certain types of food are processed at specific places in the digestive tract and that all digestion is not confined to the stomach as popularly believed. Some foods, indeed, are processed in more than one place in the tract.

■ The purpose of this exercise is to classify some common foodstuffs as starches, sugars, fats, or proteins, and to correlate their chemical composition with the location where they will be broken down in the human digestive tract.

MATERIALS

Iodine solution (dilute)
Benedict's solution or sugar test paper
Bunsen burner with hot water bath
Biuret reagent
Starch suspension
Dextrose (glucose) solution, 5%
Fat (trimmed from meat), uncooked
Egg albumin
Unknowns: A, B, C, D
Miscellaneous foods: bread, uncooked meat, skim and whole milk, banana, potato, etc.
Five test tubes

PROCEDURE

The first procedure will be to determine which of the reagents can be used to test for starches, sugars, proteins, and fats.

1. Select four test tubes. Pour 3 ml of starch solution into Tube 1; 3 ml of glucose solution into Tube 2; 3 ml egg albumin (protein) into Tube 3; and place a small piece of uncooked fat into Tube 4.

To each of these four tubes add one drop of dilute iodine solution and notice the color formation, if any. In which of the tubes is there a striking color change? (1) For which of the four types of foodstuffs is iodine a test? (2)

2. Wash the tubes used above and prepare the four test tubes using new samples of the same materials, just as you did in step 1. For the Benedict's test, boil each material vigorously for two minutes in 5 ml of Benedict's solution or place the test tubes containing Benedict's solution in boiling water for three minutes and then allow each to cool. (CAUTION: *Shake the tube while heating to prevent the tube's cracking.* Do not have the mouth of the tube facing you or anyone near you. The hot material may boil violently and shoot out of the tube onto someone.) Record any color change noticed after heating. (3) For

what is Benedict's solution a test? (4) (If sugar test paper is used instead of Benedict's solution, add distilled water to each of the materials, tear off a few centimeters of the paper, and dip one end into the solution. Compare the color of the paper with the color chart provided with the test paper, and answer Questions 3 and 4 above.)

3. Again prepare the four test tubes with fresh material as in step 1 above. Add water to a depth of about 2.5 cm. Also prepare a fifth tube with 2.5 cm of water only. Add 10 drops of biuret reagent to all five tubes. Record any color changes. (5) With what does the biuret reagent normally react? (6) What is the purpose of the test tube of water in this test? (7)

4. As fats do not dissolve in water, you do not add water to the food samples when you test for fat. If the food is a solid, rub it on a piece of white paper or, if the food is liquid, pour a sample onto a piece of paper. Allow the paper to dry. Then hold the paper up to the light. A more or less permanent translucent spot indicates the presence of fat. Testing each of the four foods in this way, which one contains fat? (8) When a food contains a very small amount of fat it cannot be detected by the method just described. To test foods for minimum amounts of fat, a fat-soluble liquid such as ether is mixed with the food in the test tube and the mixture is shaken. The small amount of fat in the mixture will dissolve in the ether. Because fat is lighter than water and rises to the top, it can be poured onto a piece of paper. The ether evaporates rapidly, leaving the fat on the surface of the paper. Then test for translucency. (CAUTION: *If ether is used do not use it in a closed room nor in a room with an open flame. Ether is highly explosive.*)

You have now tried tests for four substances: starch, sugar, protein, and fat. On the classroom blackboard prepare a chart listing types of food down the left side and listing sugar, starch, protein, and fat in four columns along the top. Test the foodstuffs for these four nutrients and record your data. Do any of the foods seem to contain only one type of nutrient? (9) How many kinds of nutrient are found in one foodstuff? (10) In skim milk and banana, which nutrients seem to be absent? (11) In what way does whole milk differ from skim milk in nutrients? (12)

Obtain an unknown food sample from your instructor and determine its nutrient makeup by the use of the above techniques. Record your results on the chart on the board.

For each of the foods tested, on the basis of its starch, sugar, protein, or fat content, list the parts of the digestive tract in which it will be broken down. (13) Consult the table Human Digestion, shown before, for the necessary information.

On the basis of the above information, what foods would be affected least by not chewing food thoroughly? (14) If starches were not thoroughly chewed in the mouth and mixed with saliva would they be digested? (15) If proteins were not completely digested in the stomach would they be digested at all? (16) Of the four groups of nutrients tested, which would be the last to be digested in the human digestive tract? (17)

Which foods will be most readily digested—those thoroughly chewed or those swallowed in large chunks, and why? (18)

Remembering that the stomach is acid and the intestinal tract alkaline, what class of nutrients is evidently digested in both an acid and an alkaline medium? (19)

THE LIVING

INVERTEBRATE HEART

In the seventeenth century William Harvey spent many years studying the action of the heart and the circulation of the blood. He wrote:

I have also observed, that almost all animals have truly a heart, not the larger creatures only and those that have red blood, but the smaller and [seemingly] bloodless ones also, such as slugs, snails, scallops, shrimps, crabs, crayfish, and many others; and even in wasps, hornets, and flies. I have with the aid of a magnifying glass, and at the upper part of what is called the tail, both seen the heart pulsating myself, and shown it to many others.*

*Reprinted from Everyman's Library edition, E. P. Dutton, translated by Robert Willis from An Anatomical Disquisition on the Motion of the Heart and Blood in Animals, by William Harvey, first published in 1628.

In this lesson you will be given the opportunity to witness the working heart of a living water flea, *Daphnia*, an arthropod commonly found in freshwater ponds and lakes. A *Daphnia* is so small and transparent that, when placed under the low power of a microscope, its beating heart can be seen and studied (Figure 22-1-1).

■   The purpose of this exercise is to observe the beating invertebrate heart and through your own observations and experiments to determine its rate and the effects of changes in the environment and the effects of drugs on the rate of heartbeat.
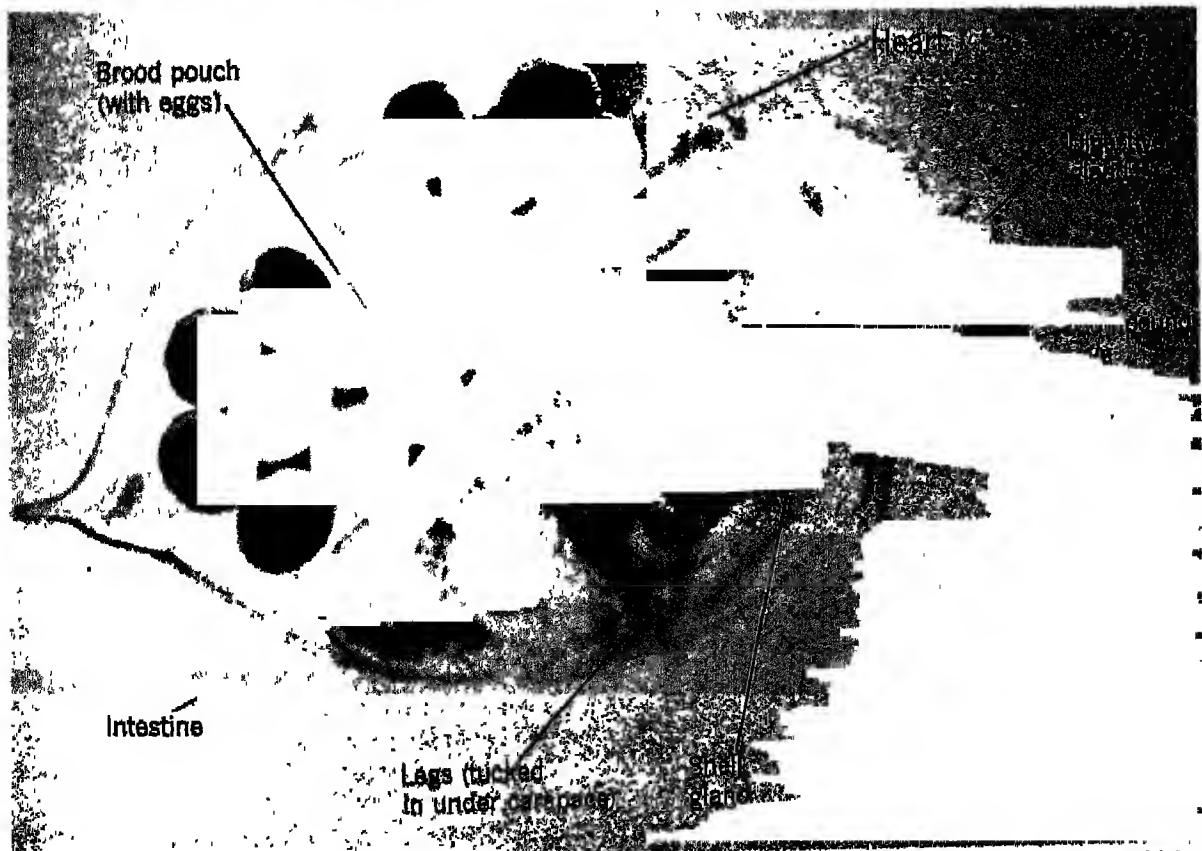
Figure 22-1-1   Internal structure of Daphnia.

George I. Schwartz

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┌─────────────────────────────────────────┐
│  MATERIALS (Parts A, B, and C)          │
│                                         │
│  A small beaker of pond water containing│
│    Daphnia                              │
│  Wide-mouthed  medicine-dropper pipette │
│    (for "fishing out" a single Daphnia) │
│  Slide and cover glass                  │
│  Some bristles or pieces of broken cover│
│    glass to support the cover glass and │
│    prevent Daphnia from being crushed   │
│    (CAUTION: If you use pieces of glass,│
│    handle them with a pair of forceps,  │
│    not with fingers)                    │
│  Thermometer                            │
│  Large beaker of ice, water, and salt   │
│    into which the small beaker containing│
│    Daphnia will fit in order to lower its│
│    temperature                          │
│  Similar beaker containing warm water   │
│    (36° C)                              │
│  Small dishes into which to transfer the│
│    cooled or warmed Daphnia             │
│  Watch with a sweep second hand         │
│  Stereoscopic dissecting microscope     │
│  Dexedrine sulfate solution             │
│  Chlorpromazine solution                │
│  5% alcohol                             │
│  Graph paper                            │
└─────────────────────────────────────────┘
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PROCEDURE

Work in teams of two students, following the special instructions below.

Part A:
The Heartbeat
at Room Temperature

Student A: Take the temperature of the water in the container of *Daphnia* and record it in your notebook.

Student B: Place one of the *Daphnia* on a slide in a drop of water. Place three or four pieces of broken cover glass or bristle in the drop and cover with a cover glass. Place the slide under the microscope and bring the heart into focus.

Student A: Look through the microscope at the beating heart and get ready to count heartbeats.

Student B: Look at the watch (with sweep second hand) and, when ready, say, "Go."

Student A: Begin to count the heartbeats using the method outlined for you by your teacher.

Student B: At the end of 15 seconds, say, "Stop."

Student A: Count the dots on your piece of paper and give the count to your team-mate.

Student B: Multiply the count by 4 (15 seconds × 4 = 1 minute) and record the calculation.

Repeat this procedure at least three times. Calculate the heartbeats per minute and take the average of your three trials. Record this average count in your notebook.

Part B:
The Heartbeat
at Other Temperatures

Raise or lower the temperature of the water in the beaker containing *Daphnia* by placing the small beakers containing *Daphnia* in either the larger beaker of warm water or in the beaker containing ice, water, and salt.

Place the bulb of the thermometer in the small beaker of *Daphnia* and very gently (so as not to harm the *Daphnia*) rock the small beaker to make the temperature of the water the same throughout the beaker. Watch the thermometer until the desired temperature is reached. Your particular temperature will be assigned to you as follows.

Two teams determine the rate of heartbeat at 30° below room temperature by transferring the *Daphnia* and some of the cooled water to a previously cooled dish and observing the *Daphnia* under the dissecting microscope. Be sure to make the observations before the temperature rises.

Two teams determine the rate of heartbeat at *25° below room temperature*.

Two teams determine the rate of heartbeat at *20° below room temperature*.

Two teams determine the rate of heartbeat at *15° below room temperature*.

Two teams determine the rate of heartbeat at *10° below room temperature*.

Two teams determine the rate of heartbeat at *5° below room temperature*.

Two teams determine the rate of heartbeat at 36° C.

Each team is to enter its findings in a table on the blackboard patterned after the one following.

Temperature	Average Heartbeats per Minute	Initials of Team Members

When all the data are complete, copy them from the blackboard into your own data book.

Using graph paper make a graph of the data, plotting rate of heartbeat along the horizontal axis against temperature along the vertical axis. Did all members of the class record a similar rate of heartbeat at room temperature? (1) At lower temperatures? (2) At higher temperatures? (3) How do you account for this? (4)

From your graph, what effect does lower temperature have on heartbeat? (5) Is there an equal difference between each 5° drop in temperature, or is there a temperature at which the drop is more pronounced? (6) What effect did higher temperatures have on the heartbeat? (7)

Part C:
The Heartbeat and Chemical Compounds

There are a number of substances known as tranquilizers, which are believed to quiet the nerves. One of these is chlorpromazine. Other substances known as stimulants have an opposite effect. Dexedrine sulfate is one of these. Here are some investigations you can make. Add to separate mounts of *Daphnia*, chlorpromazine solution and dexedrine sulfate solution, instead of water.

What is the effect of chlorpromazine on the heart rate of *Daphnia*? (8) Do *all* tranquilizers have the same effect? (9) What is the effect of dexedrine sulfate on the heart rate of *Daphnia*? (10) What is the effect of 5% alcohol on the rate of heartbeat? (11)

Could *Daphnia* be used to test the concentration of tranquilizers, stimulants, and other drugs of unknown concentration? (12) Bioassay is a technique using living organisms to determine quantities of suspected materials and even to identify them.

CAPILLARY CIRCULATION

As you will read in your textbook, William Harvey demonstrated the circulation of the blood but never saw the blood capillaries. This was left to the Italian, Marcello Malpighi, who, using the web of the frog's foot, first saw capillary circulation. In this exercise you will have the experience of observing circulation of the blood through the capillaries in the tail of a goldfish or, as an alternative if your teacher prefers, in the webbing of the frog's foot.

■ The purpose of this exercise is to see and to understand the function of the capillaries as that part of the circulatory system where exchanges of materials occur between the bloodstream and the cells of the body.

MATERIALS

Goldfish in a bowl or an aquarium
Dip net
Half of a Petri dish
Two wads of absorbent cotton (one thin and one thick)
Two halves of a microscope slide
Medicine-dropper pipette
Compound microscope or stereoscopic dissecting microscope
Millimeter ruler

PROCEDURE

Soak the thin wad of cotton and spread it on the bottom of the Petri dish toward one end. At the other end place the half-slide.

Soak the thick wad of cotton in water and have it ready for the next step.

Using your net, remove the fish from the water. Place it in the Petri dish in such a position that its head and body lie on the moist cotton and its tail on the half-slide.

Now place the thick, soaked wad of cotton over the body of the fish, and the other half-slide over the tail so that the tail is sandwiched

between the two. The set-up should now look like that in Figure 22-2-1.

If the fish flips its tail out, as it may, simply put it back between the glass slides. You may have to do this more than once during this study. Also you will have to remember from time to time to place a few drops of water (using the medicine dropper) on the cotton to keep it moist constantly.

Remove the clips from the stage of your microscope and then place the Petri dish on the stage so that the fish's tail is over the hole in the stage.

Focus the microscope on the tail. Then move the dish around until you find a part of the tail in which you clearly see the capillaries and flowing blood. The capillaries are the smallest in diameter of the blood vessels you can see here.

Look for a small artery (arteriole) at a point where it divides. The two forks of this division are the capillaries. Measure the diameter of the arteriole, and record. (1) Measure the diameter of each of the capillaries coming from the arteriole and record each. (2) Is the sum of the diameters of the capillaries greater or less than the diameter of the arteriole from which they arose? (3) In which vessels would there be the greatest amount of surface for the volume of blood flowing through, in the arteriole or in the capillaries that branch from it? (4) Is the total cross-sectional area through which the blood can pass greater in the arteriole or greater in the capillaries that spring from it? (5) On the basis of these measurements and your observations, would you expect the blood to be flowing more slowly or more rapidly in capillaries than in arterioles from which the capillaries arise? (6) Do your observations bear out your conclusion in Question 6? (7)

The small objects which you see moving through the capillaries are red blood cells. What is their shape? (8) How many of them can pass side by side through the capillaries? (9) Can you formulate a hypothesis to account for
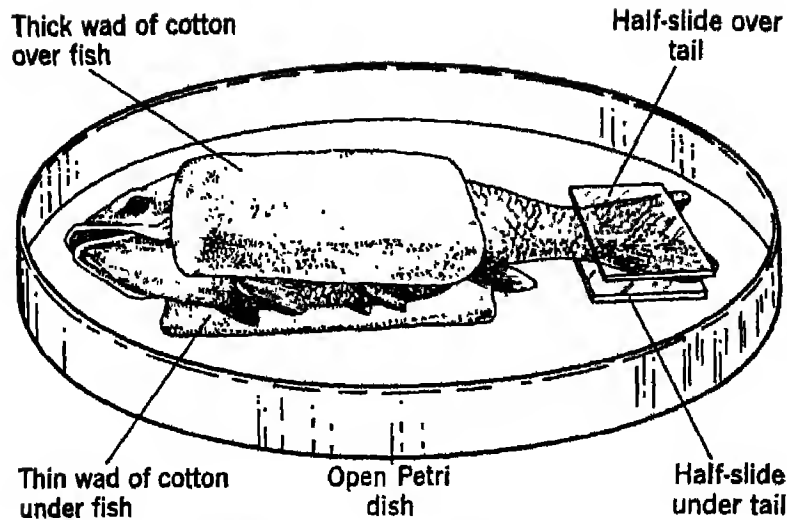
Figure 22-2-1 Preparation for observing circulation in a living fish.

any advantage to the animal in having the red blood cells pass through the capillary as they do? (10)

Trace a capillary in the direction of the blood flow until it joins with another one to form a slightly larger vessel (venule). Measure the length of a typical capillary, and record. (11) Does the blood move more rapidly or less rapidly after it flows from the capillaries into a larger venule? (12) Is the diameter of the venule the same or different from the diameter of the arteriole from which the capillaries arise? (13)

Why is it advantageous that the capillaries have as thin walls as possible? (14) Would you expect much exchange between red blood cells and the body cells in arteries or veins? Why? (15)

Measure the distance between capillaries in the tail. Would you assume that metabolism is as high in the fish's tail as it would be, for example, in some of the body muscles? Why? (16) In view of your answer to Question 16, would you assume that capillaries would be closer together in muscle than in the tail region you are observing? (17) What characteristic of raw mammalian meat (muscle) in general would lead you to believe your answer to Question 17 relates to muscles in animals other than fish? (18)

HUMAN RESPIRATION

Many of the products of metabolism can be measured. However, as the amounts of the products produced vary enormously, different units of measurement must be used for some amounts rather than for others. In this exercise we will be measuring the production of carbon dioxide in a human being. The unit we will use to measure carbon dioxide production is the micromole. To understand what a micromole is, the following explanation may be helpful.

As you have learned, every element with which we deal has an atomic weight. The element hydrogen (H), for example, has an atomic weight of 1. Oxygen (O), has an atomic weight of 16. These atomic weights contribute to the weight of a molecule, which is the sum of the atomic weights of the atoms in the molecule. For example, the molecular weight of the hydrogen molecule (H_2), is 2 and the molecular weight of the oxygen molecule (O_2) is 32. A mole of any substance is its molecular weight in grams. Thus, 2 grams of hydrogen is a mole of hydrogen. Thirty-two grams of oxygen (O_2) is a mole of oxygen. As you can see, the molecular weight of carbon dioxide (CO_2) would combine the atomic weight of 12 for carbon (C) with two atomic weights of 16 for each oxygen atom, so that a CO_2 molecule will have a molecular weight of 44. A mole of CO_2, therefore, would weigh 44 grams.

However, because 44 grams of carbon dioxide is a very considerable volume of the gas, it is not convenient to measure its production in moles. Micromoles, which are millionths of a mole, are used instead.

In this exercise we will use an indicator, which is a chemical whose color changes with a change in the pH and whose color change indicates a specific pH range. The indicator we will use is a phenolphthalein solution, which turns pink in the alkaline pH range.

Using the indicator, you will attempt in this exercise to determine the amount in micromoles of the carbon dioxide in the breath you exhale.

■ The purpose of this exercise is to determine the amount of carbon dioxide produced by your metabolism and to compare it with the metabolism of your fellow students, and thus to gauge the rate of respiration of your cells and in general those of the human body.

MATERIALS

Graduated cylinder
Flask or large bottle
0.04% sodium hydroxide (NaOH) solution
Phenolphthalein solution
Soda straw or glass tubing
10-ml graduated pipette or burette tube
Watch with a second hand

PROCEDURE

Measure 100 ml of tap water in a graduated cylinder and transfer it to the flask or bottle.

Add 3 to 5 drops of phenolphthalein indicator to the flask. Do you notice any color change? (1)

As most tap water tends to have a neutral pH, add sufficient drops of the 0.04% NaOH solution to obtain a pink color. Is the solution now alkaline or acid as indicated by the pink color? (2)

Blow through a soda straw or glass tube into the water solution *for exactly one minute. Breathe in normally,* but exhale through the tube. Start counting the time with an inhalation. Blow gently so that water will not splash from the flask or bottle. What changes do you observe in the color of the solution? (3) What does this indicate about the pH? (4) What compound is formed when CO_2 is bubbled through water? (5) How many seconds did it take for the color to change? (6)

Slowly and carefully, drop by drop, add the 0.04% sodium hydroxide (NaOH) solution to the solution in the flask or bottle by using either a 10-ml graduated pipette or burette tube. (CAUTION: *Sodium hydroxide is a strong alkali. Avoid contact with skin and*

clothing.) Swirl the water *gently* in the flask while adding the NaOH. Continue to add NaOH until you are sure the pink color will last for a minute.

Record the number of milliliters of 0.04% NaOH solution you added to turn the water pink and keep it pink for one minute after swirling.

Your teacher may want to go into the chemistry of the change with you. However, if you multiply by 10 the number of milliliters of 0.04% NaOH solution to turn the solution pink and keep it pink for one minute after swirling, the product is the number of micromoles of CO_2 which you exhaled in one minute.

Put your name on the blackboard and beside it record the number of micromoles of CO_2 exhaled by you in one minute. Other members of the class will do likewise. Do all members of the class exhale the same amount of CO_2 in micromoles per minute? (7) What is the class average of micromoles of CO_2 exhaled per minute? (8)

What is the significance of the variation in micromoles of carbon dioxide exhaled per minute? (9) Since carbon dioxide is a product of cellular metabolism, what does the amount of carbon dioxide exhaled per minute tell you about the metabolism of the individual concerned? (10)

On the basis of your observations, formulate a hypothesis that will account for the variations in the amount of carbon dioxide in micromoles exhaled per minute by various members of the class. (11)

Devise a simple experiment to test your hypothesis. This experiment may include one class member who has been at rest for five minutes before performing the test and another who has been exercising vigorously for a minute or two before the test. Are there differences between the micromoles of carbon dioxide produced by these two class members per minute? (12) Does this observation substantiate your hypothesis? (13)

You may have heard of the basal metabolism test which determines how rapidly the resting body uses oxygen. Can the production of carbon dioxide be considered a type of basal metabolism test? (14)

HUMAN KIDNEY FUNCTION

In previous exercises on animals you have learned that excretory organs are not found in animals whose surface area is large enough and exposed enough to serve as a sufficient excretory surface. However, special excretory organs are present in such animals as the planarian, whose surface area is too small to serve effectively as an excretory surface, or in animals that consume food containing large volumes of nitrogenous compounds and have skin or scales or an exoskeleton that cover the living cells of the body surface.

The function of the excretory system in the human being is to maintain a constant level of chemical substances in the blood. In other words, it is a mechanism for homeostasis of the body fluid.

Figure 24-1-1 shows the human excretory system and its major parts in relation to the circulatory system. The actual unit, however, which does the work of selecting those sub-

stances to be excreted is the nephron. Each kidney contains countless nephron units, each of which is composed of a capsule (Bowman's capsule) surrounding a little tuft of capillaries called a glomerulus, and a long coiled tubule leading from Bowman's capsule to the collecting ducts of the kidney. Figure 24-1-2 shows the relationship of the circulatory system to Bowman's capsule and its tubule. As shown in this drawing, material first passes out of the arterial blood into the Bowman's capsule. This filtrate proceeds down the kidney tubule. In the area where the kidney tubule passes through a capillary network, useful materials and much water are reabsorbed into the bloodstream. There is additional excretion of wastes into the filtrate, and the filtrate thus becomes the concentrated urine that is excreted.

■ The purpose of this exercise is to understand the role of the kidney in excretion and in maintaining the homeostatic condition of the blood.
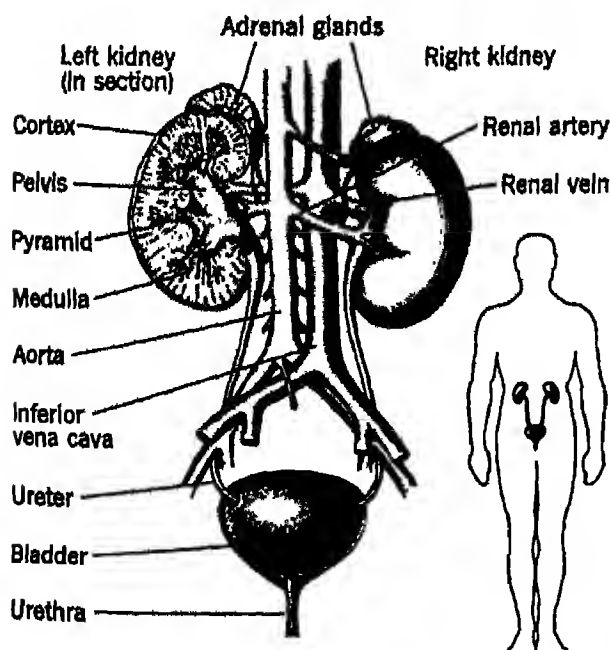
Adrenal glands
Left kidney (in section)
Right kidney
Cortex
Pelvis
Pyramid
Medulla
Aorta
Inferior vena cava
Ureter
Bladder
Urethra
Renal artery
Renal vein

From Life: An Introduction to Biology by Simpson, Pittendrigh and Tiffany, © 1957, by Harcourt, Brace & World, Inc. and reproduced with their permission.

Figure 24-1-1 Human excretory system.

MATERIALS

One bottle for urine sample (pint bottle) with cork or cap
A few crystals of thymol as a preservative
Twenty test tubes and corks
Glacial acetic acid
Methyl alcohol solution of xanthydrol
Pipette for transfer of samples
10% silver nitrate solution
Test paper for urine glucose
Bunsen burner
Large beaker (750 ml)
Graduated cylinder
Bial's reagent
1.0% ferric chloride ($FeCl_3$) solution
0.1 M urea solution
0.1% glucose solution
0.1% sodium chloride (NaCl) solution
0.25% pentose sugar solution

Proximal convoluted tubule
Glomerulus
Bowman's capsule
Afferent arteriole
Efferent arteriole
Distal convoluted tubule
From renal artery
Capillary net
Collecting tubule
KIDNEY
To renal vein
Renal vein
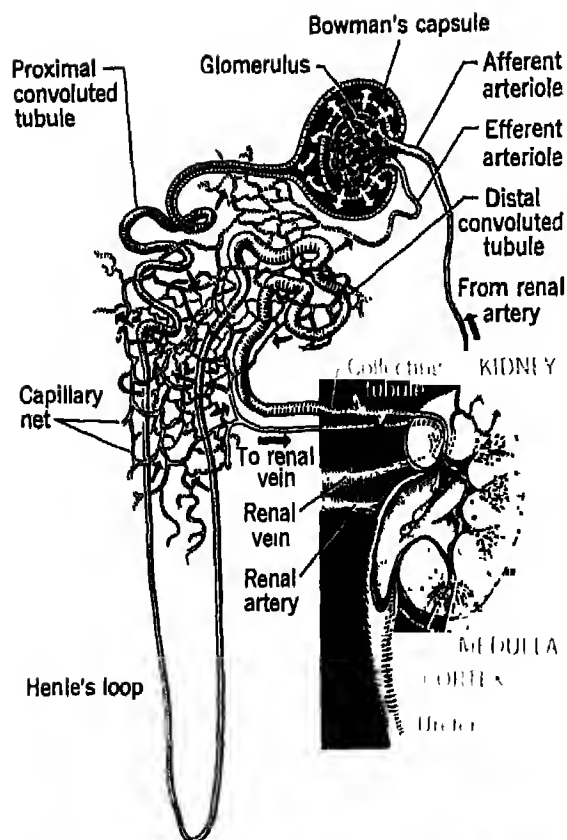Renal artery
Henle's loop
MEDULLA
CORTEX
Ureter

From Life. An Introduction to Biology by Simpson, Pittendrigh and Tiffany, © 1957, by Harcourt, Brace & World, Inc and reproduced with their permission.

Figure 24-1-2 Detail of a human kidney tubule.

PROCEDURE

Study the data in the following table, Absorption in the Kidney. This table includes data for seven substances found in the blood. It gives the changes in their blood concentrations from the time they enter the glomerulus until they leave in the renal vein, and also their concentrations in the urine. The concentrations of the substances changes as the urine is concentrated and moves toward the pelvis of the kidney. The figures represent relative amounts of substances found in 100 liters of water and 1 liter of urine. For each of the seven substances in the blood, calculate the percentage that is reabsorbed from the tubule by the capillary network.

Are all substances reabsorbed in equal amounts? (1) What two substances does the kidney remove from the blood in greatest quantity? (2) What substances are returned to the blood from the tubule in greatest amount? (3) How would these data be changed by a large intake of water? (4) By a high-salt content diet? (5) By a low-protein diet? (6)

In order to test the effect of food intake on urine composition, different students or teams of students will be asked to volunteer for different diets for one day prior to the laboratory tests, as follows:

Diet 1. *High Protein.* For this diet avoid starches, fats, and sugars. Eat meat and other high-protein foods.

Diet 2. *Salt-free.* Drink a great deal of water but make sure that the rest of your diet is as free of salt as possible. Salt-free foods can be purchased at most grocery stores.

Diet 3. *High glucose.* In addition to your regular diet, which may be cut slightly, eat several candy bars.

Diet 4. *High pentose.* Substitute for part of your regular diet, fruits such as bananas, plums, etc. (Remember that these foods also contain hexose sugars.)

Diet 5. *Regular diet.* Eat what you usually do.

Sample menus (weights and volumes are approximate. You may eat more of a measured item than indicated.)

ABSORPTION IN THE KIDNEY

Substance	A. Arterial Blood* (Afferent Arteriole) and Filtrate in Bowman's Capsule	B. Venous Blood (Renal Vein)	C. Urine (Collecting Tubule)	% Reabsorbed from Tubule (B/A)
Water	100 l	99 l	1 l	
Chloride	370 g	364 g	6 g	
Glucose	70 g	70 g	0 g	
Urea	30 g	10 g	20 g	
Uric acid	4 g	3.5 g	0.5 g	
Calcium	10 g	9.85 g	0.15 g	
Creatinine	1 g	0 g	1 g	

* Amount necessary to produce 1 liter of urine.
Data from J. A. Moore. 1957. *Principles of Zoology.* Oxford Univ. Press.

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HIGH-PROTEIN DIET

Breakfast	Lunch	Dinner
Fruit—1 orange	Meat—120 g	Meat—120 g
Cereal—whole wheat with milk and sugar	Potato	Potato—100 g
Eggs	Salad—100 g	Bread—whole wheat
Bread—whole wheat	Bread—whole wheat	Butter or margarine
Butter or margarine	Butter or margarine	Dessert
	Milk—250 ml (8 oz)	Milk—250 ml (8 oz)

LOW-SALT DIET (Everything unsalted)

Breakfast	Lunch	Dinner
Citrus fruit	1 egg or unsalted cottage cheese	Fresh meat, fish, or fowl
Unsalted cereal with a low-sodium milk substitute and sugar	Potato, rice, etc., unsalted	Potato
Egg	1 vegetable	Vegetable
Unsalted bread and butter	Salad—no dressing	Bread and butter
	Bread, butter, fruit	Dessert
	Low-sodium milk substitute	Low-sodium milk substitute

On awakening (the day before the laboratory experiment), void the overnight urine. Do not collect it. For breakfast, lunch, and dinner stay on the prescribed diet. Collect the urine sample upon awakening the second day. Place a few crystals of thymol in the bottle to act as a preservative. If possible, keep this 24-hour specimen refrigerated.

Mark the bottle with the type of diet you have followed and take it to class for testing. If several students have followed the same diet, urine samples may be pooled for the following tests. For each of the tests use a sample of urine in one test tube and, in a second test tube, add a known compound (urea, glucose, sodium chloride, pentose sugar) so that you

can compare your result with a positive test. For example, when you run the test for glucose, put a sample of 0.1% glucose solution in the other test tube to act as a control on the materials and your procedure.

Test 1. *Urea.* Dilute 1 ml of urine with water to make 500 ml of solution. Transfer 5 ml of this diluted urine to a test tube and add 5 ml of glacial acetic acid and 0.5 ml of a methyl alcohol solution of xanthydrol. Cork the tube and shake it vigorously. Allow it to stand for at least 1 hour. Formation of large, loose clumps indicates that urea was present. These are stable, so the tube can be left overnight if necessary, before making observations.

Test 2. *Chloride.* Dilute 1 ml of urine with water to make 500 ml. Transfer 5 ml of diluted urine to a test tube and add a few drops of 10% silver nitrate solution. A cloudy white precipitate indicates that a chloride is present.

Test 3. *Glucose.* Immerse a 4 cm piece of urine glucose (sugar) test paper in the urine sample. Remove the paper and allow 1 minute for color to develop. If glucose is present, the color of the paper can be matched with the color chart on the test paper container to obtain the approximate percentage of glucose present. If more than 0.5% glucose is indicated, wait another minute to be certain of full color development before matching colors. If the instructions with the paper you use differ from those given here, follow the directions provided with the test paper.

Test 4. *Pentose sugar.* To 2 ml of urine in a test tube add 2 ml of Bial's reagent. Gently warm the tube over the Bunsen burner and add a few drops of the 1.0% solution of ferric chloride. A deep green color indicates pentose sugar.

On the blackboard write the results for each test and diet. What are the effects on the urine of the high-protein diet (7), the salt-free diet (8), the high-glucose diet (9), the high-pentose diet (10), in comparison with a regular diet? (11) Would you expect blood samples from people on a high-protein diet and on a high glucose diet to be similar? (12) Why? (13)

What does a positive urine glucose test indicate about blood glucose level? (14)

Write a brief paragraph describing the effectiveness of the kidney in maintaining homeostasis in the blood. (15)

ANIMAL BEHAVIOR

All animals have some characteristics of behavior that are unique to the species. If you watch a spider build its web, or a wasp capture a grasshopper and bury it in the ground, or a caterpillar spin its cocoon, many intriguing questions arise about the origins and patterns of behavior.

Questions concerning the origin of specific behavioral characteristics are under constant study by scientists. Some behavior appears to be inborn (innate) while other activities seem to be learned or acquired later in the life of the organism. Because problems of inborn or inherited behavioral patterns and learned or acquired ones have certain common features in all animals, much of the study of behavior in the human being is based on experiments with lower animals, including insects.

In earlier exercises dealing with living animals you became acquainted with some features of their behavior—the trial-and-error movements of a paramecium and the tactic responses of worms to light, chemicals, heat, and other stimuli.

■ The purpose of the present exercise is to study some of the behavioral characteristics of two kinds of insects, an adult fly (a fruit fly) and a larval form (caterpillar) of either a moth or a butterfly. The behavior observed in these insects will then be compared with some simple examples of human behavior that most people may not even think of as behavior. In conducting these studies of man and insects, keep in mind the possibility that in some respects the behavioral patterns may be similar.

PROCEDURE

Part A:

Reactions of Fruit Flies to Light

Hold the test tube of fruit flies horizontally about 30 cm (1 ft) from a glowing light bulb. Do the flies tend to move toward or away from the light? (1) When handling the tube of flies, slowly turn it vertically and then horizontally, or at an angle, but do not jar or tap the tube or otherwise disturb the flies. Why? (2) Change the position of the tube and hold it 100 cm from the light. Note the flies' reaction. Is it the same as at 30 cm? (3) By moving the tube closer to and farther from the light, determine the optimum distance at which the flies respond to the stimulus of the light. (4) What is the minimum distance? (5) What is the maximum distance? (6)

What do the above observations indicate about fruit fly behavior in relation to the brightness or intensity of the light? (7) Using colored light bulbs or colored cellophane, determine the reaction of flies to lights of different colors. Do the flies react equally well to light of any color? (8) Does the position of

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MATERIALS (Parts A and B)

Test tube containing several fruit flies
One living caterpillar
Plant material *known* to be a source of food for the caterpillar and some plant material known *not* to be a source of food for this caterpillar
Food coloring
Liquid chlorine bleach (Clorox or a similar brand)

Rubbing alcohol
Deodorant
Paraffin
Light bulbs of different colors or colored cellophane wrapped over white light bulbs
Sources of heat and cold
Paper with a circle of 15-cm diameter

the tube determine in any way in what direction the flies tend to move? (9)

In addition to emitting light, the electric bulbs also emit heat. What effect do you suppose the emission of heat may have on your observations concerning fruit flies and light? (10) Devise a simple experiment that will test the reaction of the flies to heat or cold alone but not to light. Do the flies react to different temperatures by their direction of movement? (11)

Do the reactions observed in the flies seem to be inborn or learned? (12) How could you determine whether the reactions exhibited by the fruit flies are learned or inborn? (13)

Part B:
Food Reactions of a Caterpillar

On a piece of paper marked with a 15-cm circle, arrange four items of possible foods of the caterpillar at opposite points around the circle. Use one piece of a plant normally eaten by the caterpillar, and three pieces of different plant materials known not to be a source of food for this insect. Place the caterpillar in the center of the circle. How does the caterpillar move in response to the presence of its food? (14) What factors present in this experiment tend to complicate the results and make it less accurate? (15)

Continue your observations by altering the distances of the food materials from the center of the circle in order to determine the distance through which the larva is able to locate its specific food. Rotate the positions of the various food materials during the course of the observations. What is the point of doing this? (16)

Prepare a chart with the four food items listed across the top and the distance from the center of the circle listed down the left side. Record the number of times the caterpillar moves to each food source and the distances through which it moves.

When the larva can locate its food in at least 80% of the trials, assume that distance to be optimum. What is this distance? (17)

In nature, what would be the effect of removing a caterpillar a considerable distance from its food source? (18)

How do you suppose a caterpillar locates its food—by its odor, color, or shape? Attempt to answer these questions by the following experiments.

Color. Dye the plant materials different colors with food coloring and attempt to determine if color plays an important role in food location. Give the results of this test. (19)

Odor. To determine if odor plays a part, soak bits of the plant food material in liquid chlorine bleach or rubbing alcohol to disguise the natural odor. You might try several brands of standard deodorants. Try dipping the food in melted paraffin. Are any of these methods of altering the normal odor of the plant successful in preventing the larva from locating its food? (20)

Shape. Is the shape of the food material of possible importance? Devise an experiment that will measure the caterpillar's ability to locate its food by sight alone, irrespective of color and odor. Does food shape seem to determine the way in which the caterpillar finds its food? (21)

On the basis of your experiment, does this particular caterpillar locate its food primarily by odor or sight? (22) Do these experiments adequately demonstrate the method by which this particular caterpillar is able to locate its food? (23)

Part C:
Reflex Actions in Human Beings

Actions that occur automatically and without conscious effort are called **reflex actions** and are brought about when certain nerves are stimulated. We are born with some of these automatic behavior abilities and acquire others later in life. Natural reflexes are sometimes difficult to distinguish from acquired reflexes, but the natural ones are not dependent on past experience or training, like the acquired ones.

Following are a few experiments involving reflex action that can be carried out in the classroom. Do these experiments in pairs with one student as a subject, the other as an observer.

The Knee Reflex. The subject sits on the desk top or on a chair, with one leg crossed over the other so that his foot is off the floor. The observer then with the edge of his open hand gently strikes the subject's knee just below the kneecap. If the knee is struck in the proper place a reflex action occurs. What is the response? (24) Does it vary with the intensity of the stimulus? (25) Is this reflex equally pronounced in all students in the classroom? (26) Do you believe this reaction to be learned or natural? (27) Does the subject indicate that thought was necessary in order to perform the

reflex? (28) By conscious effort can the individual suppress the reflex? (29)

The Eye Reflex. The subject sits on the desk or in a chair with the observer directly in front of him. The subject looks directly at the observer. The observer then suddenly raises his hands and claps them in front of the subject's face. Be careful not to come too close! What is the subject's reaction? (30) Is this a learned or an innate bit of behavior? (31) Repeat the clapping several times. Does the subject seem to be able to *learn* not to blink? (32)

The Pupil Reflex. The subject sits on the desk, and the observer watches the pupils of both of the subject's eyes. With both eyes open, the subject covers his right eye with his right hand, blocking out as much light as possible. After fifteen seconds the subject uncovers his eye and the observer compares the size of the pupils of each eye. What has happened to each pupil? (33) Does the subject report that he can feel that change taking place? (34) The subject now uncovers both eyes and the experiment is repeated. Do both eyes respond in the same fashion as the right eye previously did? (35)

Is there any similarity between the reflexes observed in the human being and the responses of the flies and the caterpillar? (36)

CONTROL OF

MUSCLE CONTRACTION

The muscle of the heart is called cardiac muscle. The muscle of the stomach is called smooth muscle. Both types of muscle are controlled by the nervous system, in particular by the part of the nervous system called the autonomic (unconscious) nervous system, so-called because it is not under the conscious control of the will. You cannot start or stop your heart at will, for example. The autonomic nervous system can be divided into two parts—the parasympathetic and the sympathetic—which have opposite effects from each other. This exercise will demonstrate responses of cardiac and smooth muscle to stimulation of the two types of nerves.

Remember that the sympathetic nervous system secretes an adrenaline-like substance and the parasympathetic nervous system secretes an acetylcholinelike substance. Therefore, the use of acetylcholine and adrenaline should respectively resemble the stimulation of the nerves of one system or the other.

■ The purpose of this exercise is to observe the regulation of contraction of both types of muscle and to correlate it with the nervous system secretions.

MATERIALS

Pithed frog
Ringer's solution
Four small Petri dishes
Needle, scissors, forceps
Four medicine-dropper pipettes
Adrenaline solution, 1:10,000 aqueous
Ice
Acetylcholine solution, 1:10,000 aqueous
Source of heat (Bunsen burner or substitute)
0.95% sodium chloride (NaCl) solution
Thermometer

PROCEDURES

Take a pithed frog and with scissors open its body cavity (Refer to Exercise 20-3 as necessary). Locate the heart; cut open the pericardial sac and locate all blood vessels leading to and from the heart. With scissors, cut the blood vessels which hold the heart in the body cavity and with forceps remove the heart to a Petri dish containing Ringer's solution.

Remove the stomach by cutting with scissors at the bottom of the esophagus and just below the pylorus. Place the stomach in the dish with the heart and gently rinse away blood and other waste materials.

Now put both organs into fresh Ringer's solution and count the heartbeats for 45 seconds. What is the rate of heartbeat in Ringer's solution? (1) Does the stomach exhibit any activity when observed for 30 seconds? (2) Compare the activity of the stomach and the heart while in Ringer's solution. (3)

Transfer both the heart and the stomach to a Petri dish containing a solution of three-quarters Ringer's and one-quarter adrenaline solution. Observe the heartbeat for 45 seconds. What is the rate under the influence of adrenaline? (4) Observe the action of the stomach in the adrenaline solution. How does it differ from what it was when in the Ringer's solution? (5)

Transfer both organs to fresh Ringer's solution to rinse them. Then transfer the tissues to a solution containing one-quarter acetylcholine and three-quarters Ringer's solution. Again observe the heartbeat for 45 seconds. What is the rate now? (6) Observe the activity of the stomach and record the force and rate at which it contracts. Compare the action of the stomach now to its action in Ringer's and in adrenaline. (7)

Take both organs from the acetylcholine-Ringer's solution bath and place them in a dish of Ringer's solution for a 2-minute rinse.

Transfer the two organs from the Ringer's bath to a dish containing Ringer's solution heated to about 30° C. Observe the heartbeat for 45 seconds. What is the influence of the 30° C temperature on the rate of heartbeat? (8) Observe the force and rate of contractions of the stomach. Do they increase or decrease under the higher temperature? (9)

Transfer the organs to a saline rinse (room temperature) for 45 seconds.

Then transfer the tissue to Ringer's solution chilled by placing the dish on ice. (Do not add ice to the solution.) Count the heartbeat for 45 seconds. How does it differ from the heartbeat at 30° C? (10) Observe the force and rate of stomach contractions. In what way are they different from those at 30° C? (11)

Transfer both tissues to a dish of 0.95 NaCl solution. Count the heartbeat for 45 seconds. In what way is it different from the rate of heartbeat in Ringer's solution? (12) Similarly,

observe the stomach contractions and compare them to the stomach contractions as seen in Ringer's solution. (13)

Do the heart and the stomach respond in the same way to adrenaline? (14) To acetylcholine? (15) On the basis of what you know about the relation of these chemicals to the nerves, would stimulation of the sympathetic nervous system increase or decrease the rate of the heartbeat? (16) Increase or decrease stomach contractions? (17) What would be the effect of stimulation of the parasympathetic nervous system on these two organs? (18)

Do both the heart and the stomach respond in the same way to the 30° C temperature? (19) To the ice? (20)

Why is innervation from two types of nerves necessary for control of activity of the heart and the stomach? (21) Which of the two drugs tested would probably be used in cases of heart failure? (22)

EFFECTS OF

REPRODUCTIVE HORMONES

The male hormone, testosterone, is produced in the interstitial cells of the testes. It controls the development of the sex organs (primary sex characteristics). It also has an effect upon the development of the secondary sex characteristics — for example, in poultry, on male coloring of the plumage, certain anatomical differences such as comb development in male chickens, and male behavior patterns. Applications of testosterone to young male chickens can elicit early development of the comb and precocious male behavior, including crowing. It is testosterone which is responsible for male sex behavior. Females injected with testosterone often develop secondary male sex characteristics.

■ The purpose of this exercise is to determine the effects of testosterone, applied in different concentrations to male chicks, in respect to the development of combs and male behavior patterns, including crowing; also, to compare the effect of this hormone when applied externally and when injected subcutaneously.

MATERIALS

Three or more one-day-old cockerels for each team

Housing for chicks

Aqueous solution of 3 different dyes

1-ml hypodermic syringe

Hypodermic needle (No. 27 for water solutions of testosterone; No. 25 for oil)

Millimeter ruler

Scissors, fine-pointed

Testosterone (in oil or water). 5 ml of each of three concentrations, as follows:

10 mg per ml solution

30 mg per ml solution

50 mg per ml solution

PROCEDURE

Each team will divide its chicks into three groups, each group consisting of one or more birds. Mark each chick by swabbing some dye either on the down of the head or on the back between the wings. Use a different color to indicate each type of treatment to be used. One group will be used as a control; a second will receive testosterone injected subcutaneously; and the third will have the hormone solution applied externally to the comb. With a small pair of scissors remove the down feathers around the base of the combs of this last group.

Team A (designated by the teacher) will use the 10-mg per ml solution of testosterone on its chicks. Team B will use the 30-mg per ml solution on its chicks; and Team C will use the 50-mg per ml solution. Each team will complete the following procedure.

It will be necessary to have some means of measuring comb development in the chicks. To do this, measure in millimeters: 1. the length of the comb, 2. its height at the highest point, and 3. its greatest width at the base. For this last measurement, hold the ruler directly above the comb and look down to each side of the base for an estimate. Multiply these three measurements to obtain what we will call the comb index; that is, L × W × H = the comb index. Determine the comb index for each bird, including those in the control group. Add the indexes for each of the birds of each group. Then divide the sum by the number of birds to get an average index for each group. Be sure to keep adequate records of these data on a chart similar to the sample data sheet which your teacher will show you.

Into all chicks of Group I, inject subcutaneously 0.1 ml of testosterone of the concentration assigned to your team. This should be injected into the skin near the base of the neck or in the crop region which is located anteriorly at the base of the neck. Massage the area

gently after the injection to distribute the fluid and keep it from coming out. Repeat the injections for 5 consecutive days.

Anoint the combs of each bird in Group II with 0.1 ml of the testosterone solution assigned. Try to avoid letting the material run onto the feathers. It is best for one person to hold the chick while another applies the solution. Treat each of the birds in this group on 5 consecutive days. The Group III birds are to be kept as controls.

After 5 days of daily treatment, compute the comb index for all birds, including the controls. Record all your data.

.Watch the behavior of the three groups of birds assigned to your team. Look for evidence of fighting, crowing, or overall dominance. Record your observations.

Complete your data sheet by exchanging your records with those of the other two teams, so that all three teams have complete records

for all three concentrations of testosterone. You may also wish to pool data from the entire class or even include data from other classes to increase the sample size.

What is the purpose of having a control for each team? (1) Is there a significant difference between the comb growth in the chicks injected subcutaneously and those anointed on the combs? If so, in terms of comb index, how much? (2) From the data you have gathered, summarize the effects of different concentrations of testosterone on comb development when applied subcutaneously (3), and when applied directly to the comb. (4) If the injections and anointing were to be carried on indefinitely, which chicks do you think would be the first to crow? (5)

Capons are roosters that very early in life had their testes removed. What do you think would be the effect of injecting testosterone into these older birds? (6)

REPRODUCTION AND

DEVELOPMENT IN THE FROG

The pituitary gland, which is attached to the lower surface of a vertebrate's brain, influences the activity of the other endocrine glands by means of hormones. Among these glands, it controls the functioning of the gonads by means of hormones that stimulate or inhibit the gonads. When the frog's time for egg-laying approaches in the spring, large quantities of pituitary hormones are released into the bloodstream. Reaching the ovaries, these hormones cause the eggs to be liberated into the oviducts and uteri, from which the eggs are then released into the water at the time of laying.

In the males, the testes are stimulated by the same pituitary hormones. The stimulated gonads in turn secrete hormones of their own, and these sex hormones, in some way we do not understand very well, influence the nervous system, making the frogs migrate from their winter hiding places to the edges of the ponds. There males and females unite and after several days the eggs and sperms begin to be released from the bodies of the pair simultaneously. The sperms fertilize the eggs as the eggs are issuing from the female's body.

Female frogs that lay eggs in the spring build up a new crop of eggs during the summer, when food is abundant. By late fall, her eggs are fully developed and need only the stimulus supplied by pituitary hormones in

MATERIALS

Part A
Mature live female *Rana pipiens* (approximately 8 cm in length from snout to anus)
Hypodermic syringe (2 ml or larger)
Hypodermic needle No. 25 for injecting pituitary suspension or No. 18 for injecting whole glands
Battery jars or small aquaria with weighted screen covers for holding the injected females
Pituitary suspension (or whole pituitary glands dissected out in the classroom)

Part B
Male frogs (*Rana pipiens*) 1 to 3 per student or team
Antuitrin "S" (chorionic gonadotrophin)
Medicine dropper
Slides and cover glasses
Compound microscopes
Hypodermic syringes (1-ml tuberculin size)
Hypodermic needles (No. 27 or No. 25, 2 cm long)
Giemsa stain

Part C
One mature, male frog (*Rana pipiens*)
Filtrated pond water or filtrated aerated tap water
Clean Petri or Syracuse dishes
Clean medicine dropper pipettes
Clean depression slides and cover glasses
Clean (free from formaldehyde, etc.) dissecting scissors, forceps, and scalpels
Compound microscope
Methylene blue stain
Anesthetic (ethyl ether, 2% urethane solution, or 0.1% chloretane solution)

Part D
Sperm suspension
Frog eggs from the pituitary-treated females in Part A of this exercise
Clean Petri or Syracuse dishes
Clean medicine-dropper pipettes
Pond water
Stereoscopic dissecting microscope or a good hand lens
2-3 mm wire loop or paper clip

order to be liberated from her ovaries. Ripe eggs can therefore be obtained out of season, that is, in the fall or winter, by injecting pituitary hormones into the mature female. We shall do this in Part A of this exercise.

Read all four parts of the exercise before beginning Part A.

■ The purpose of this exercise is to observe the effects of pituitary hormones on the reproductive cycles of male and female frogs. We will also examine frog gametes and fertilized frog eggs and watch their development into tadpoles and even into frogs.

Part A:
Reproductive Hormones
and Ovulation

PROCEDURE

Inject the female frog with the pituitary material. This injection should be made into the abdominal cavity, but care must be taken not to injure vital organs.

Place the recipients of the injections in separate, labeled containers with a small amount of water (about 2 cm deep) and cover with weighted screens. If the animals are kept at room temperature (approximately 23° C), ovulation should occur in 24 to 48 hours.

To test for ovulation and the presence of eggs in the uteri, grasp the frog's body in your right hand, the dorsal side of the frog against the palm and the legs extended between the thumb and the forefinger. Hold the legs with the left hand and gently bend them ventrally from the pelvis. Press gently with the right hand, "milking" in the direction of the cloaca. Eggs present in the oviduct will be "stripped" as needed. The frog should be kept in the cold (10° C or less), and the eggs will be fertilizable over a period of four to five days. A single female may produce between 2000 and 3000 eggs. The eggs will be in the metaphase of second meiotic division and ready for fertilization. Save the females for Part D of this exercise.

Part B:
Pituitarylike Hormone
and Sperm Release

The functioning of the frog testis is regulated by one or more of the pituitary hormones. One of these, luteinizing hormone (LH),

stimulates the growth of certain cells of the testis that produce testosterone. It also activates the mature spermatozoa, which commence their movements. Another hormone, follicle stimulating hormone (FSH), may be involved in the production and maturation of germ cells by the testis. These same pituitary hormones also affect the functioning of the ovary in the female. FSH stimulates the growth of follicles and the maturation of the egg cell in each enlarging follicle. LH stimulates ovulation.

Pituitary-type hormones appear in the urine of primates during pregnancy and are of placental origin. The human placental hormone resembles LH in many of its actions and is the basis of most pregnancy tests. In this exercise we shall use the hormone to produce sperms in the male frog. This is a common pregnancy test.

Male reproductive cells, or gametes, are called sperms. Flagella, fine long threads having a lashing or undulating movement, may be produced by certain sperms and make them motile. Not all organisms have motile sperms. In the sperms of frogs, and of man and mammals in general, the cytoplasm of the cell is largely sloughed off, and the nucleus forms an oval sperm head. The long sperm tail, a midpiece, and an egg-contacting structure at the forward tip of the sperm head are the principal remnants of cytoplasm. Partly because of the loss of their cytoplasm, mature sperms are among the smallest cells of the body.

PROCEDURE

Take control *cloacal smears* of your male frogs to make certain that they are not already releasing sperms. Using a medicine dropper collect the fluid from the entrance to the cloacal cavity. It is unnecessary to enter the cloacal cavity, since an adequate sample can be obtained at the outlet. Place a drop of this fluid on a slide, add a cover glass, and observe through the microscope under reduced light.

Now divide the frogs into three groups and inject the hormone as follows:

Group 1. Inject 0.1 ml of physiological saline solution containing 5 international units of hormone into the dorsal lymph sac of each frog in the group.
Group 2. Inject 0.2 ml of physiological saline solution containing 10 international units into the dorsal lymph sac of each frog in the group.

Group 3. Inject 0.5 ml of physiological saline solution containing 25 international units into the dorsal lymph sac of each frog in the group.

You may provide a control group if you wish.

Two hours after the injection take cloacal smears and examine them for spermatozoa. Draw a spermatozoon.

Record the results in the form of a table.

Part C:
Examination of Frog Sperms

We are now ready to examine frog sperms in more detail.

PROCEDURE

You will be supplied with a male frog that has been pithed. Cut open the ventral surface of the frog, being careful not to disturb the internal organs in this cutting process. The testes, which contain the sperms, will be found as two small oval bodies lying one on each side of the backbone ventral to the kidneys, about halfway back from the head. Remove both testes and put them in a Petri dish containing 10 ml of pond water. With a scalpel cut the testes into shreds and mash the pieces against the bottom of the dish. Do not use distilled water or Ringer's solution, since the sperms tend to be inhibited in these fluids and do not show motility as satisfactorily as in pond water. Allow this suspension to stand for about 10 minutes while the sperms become active. Sperms retain a degree of activity for at least 6 hours. However, their capacity to penetrate the egg ordinarily diminishes rapidly after 30 minutes. This sperm preparation may also be used in Part D.

Periodically check to see whether the sperms are motile. The major difficulties encountered in making such observations are due to overheating and drying out of the preparations. The use of depression slides and cover glasses, plus periodic fresh preparations, should help eliminate the difficulties mentioned. Describe any movement of the sperms that you see. (1)

Add 1 or 2 drops of the methylene blue stain from the stock bottle to 1 drop of the fresh sperm preparation. Do the sperms remain motile? (2) What additional parts of a sperm cell can you now observe? (3) What effect does the stain have on sperms? (4)

Part D:
Artificial Fertilization

In nature, fertilization of frog eggs is usually external, the sperms being poured over the eggs as the eggs are deposited in the water while the male and female frogs are clasped together. In this exercise the sperm suspension is prepared as described in Part C of this exercise (use the same preparation), not more than 30 minutes prior to insemination of the eggs. The eggs are obtained by inducing ovulation, as it was described in Part A of this exercise.

PROCEDURE

Put enough of the active sperm suspension into a Petri or Syracuse dish so that the bottom of the dish is just covered. Take your dish to the location of the pituitary-treated females and squeeze some eggs directly onto the sperm suspension. Avoid piling the eggs in large clumps. Make thin strips to give maximal exposure of eggs to sperms. With a clean pipette, bathe the eggs with the sperm suspension. Record the time on the container. Do not allow this preparation to become overheated. After 10 minutes, pour off the sperm suspension and flood the dish with pond water. In approximately 20 minutes pour off the water and replace it with fresh pond water. This serves to wash away the excess sperms and debris and to reduce bacterial growth. With forceps remove extra pieces of testes and other debris.

During the first waiting period of 10 minutes, make some careful observations of the eggs with a hand lens or stereoscopic dissecting microscope. Record these observations. Describe the appearance of an unfertilized egg. (5) Record the orientation of the eggs in the gelatinous mass at this time, by answering the question: Are the black surfaces of all eggs uppermost? (6) What functions does the jelly have? (7) A successful insemination is usually indicated after the first hour by two definite changes. See if you can notice these changes and record them. (8)

Turn a small group of eggs over with forceps and see if you can make out a gray crescent. In dark-colored eggs this is a lighter area appearing on one side, just above the boundary of the pigmented material and the yolk. Use a good hand lens or stereoscopic dissecting microscope for these observations. Observations
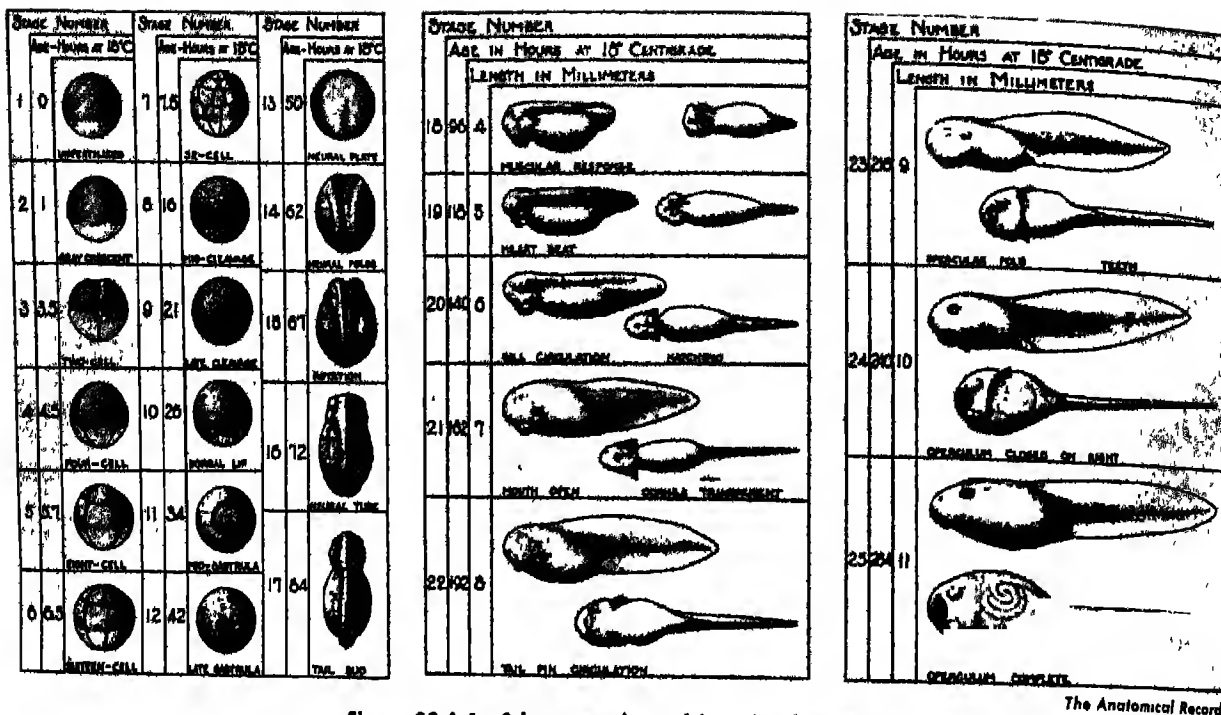
Figure 28-1-1   Schumway chart of frog development.

are easier if the egg can be held in a beam of bright light—that of a flashlight, for instance. If microscope illuminators are available these are still better light sources. It is difficult to hold one egg in the light and observe all sides of it. One method is to make a wire loop 2 or 3 mm in diameter, and to trap-the egg within this loop. You can use a paper clip with half of the bend already made.

About 2½ hours after fertilization, another important change takes place. Record this change and state its significance. (9)

You will not be able to make all these observations in one laboratory period. However, observations of eggs that were fertilized earlier in the morning can be made. Each class can add its observations if the fertilized eggs are left on the tables and the time of fertilization is recorded on each dish. Daily observations of their development can be made.

The tadpoles will need feeding after about a week. Commercial baby-food spinach is the best diet, strangely enough. The tadpoles can be kept and watered until they metamorphose into frogs.

You may want to do experiments with the eggs, such as determining the effect of temperature on rate of development.

As you observe the developing embryos, you will need to compare them with the embryos shown in the chart (Figure 28-1-1) and with the photographs of salamander embryos

in Chapter 28 of the textbook, pages 484-89.

The pictures in Figure 28-1-1 show the egg and the embryos without the jelly membranes. The first 19 stages, as you will see them, will be enclosed in jelly membranes; therefore you will see something like a halo around the embryos. You will have particular difficulty seeing the differences between stages 15, 16, 17, and 18, since the embryos will be somewhat folded in the jelly covering.

There are several points to be noticed especially in studying this chart.

a. The embryos from which the pictures were taken had developed at a constant temperature of 18° C.

b. The pictures show stages at which the embryos can clearly be distinguished. The embryos looked like this at the definite *time* they were observed, but they gradually changed from one stage to another. Many of the embryos you observe will be in an intermediate stage and will not look exactly like any of these stages.

c. The stage numbers are used for easy reference to the pictured stages of development.

d. The age in hours is the age of these stages when developing at 18° C.

e. The inscription under the pictured stage describes the appearance, new structures, or new abilities of the embryo.

f. The length of the embryos is included for the later stages.

CHICK DEVELOPMENT*

Although the chick embryo has one of the most fascinating developmental histories we know, you will probably not be able to observe all the stages mentioned below. Your teacher will provide those stages of development which are available.

■ The purpose of this exercise is to observe stages in the development of the chick, an animal with embryonic membranes, and to compare and contrast this development with that of the frog.

* Adapted from the BSCS block "Animal Growth & Development" by Dr. Florence Moog.

PROCEDURE

Part A:

The Unincubated Egg

Crack a fresh hen's egg without breaking the yolk and place its contents in a shallow dish of water. Observe the structure of the egg carefully and compare it with the internal structure shown in Figure 28-2-1. See how many parts you can identify. The embryo at this stage is very small, but can be seen as a whitish spot, called the blastoderm, on the upper surface of the yolk.

MATERIALS

Part A
Fresh hen's egg, fertilized
Shallow dish of water

Part B
One fertile egg incubated 48 hours
Fine, sharp-pointed scissors
Assortment of filter paper rings
Ringer's solution warmed to around 38° C
Petri dish or Syracuse watch glass
Fine, sharp-pointed forceps
Cotton wool
Culture dish
Paper towels
Small specimen bottles
70% alcohol
Stereoscopic dissecting microscope

Part C
One egg incubated 72 hours
Fine, sharp-pointed scissors
An assortment of filter paper rings
Ringer's solution (warmed to about 38° C)
Petri dish or Syracuse watch glass
Hand lens or dissecting microscope
Bright light
Fine, sharp-pointed forceps
Cotton wool

Culture dish
Paper towels
Small specimen jar
70% alcohol

Part D
One fertile egg incubated continuously for the past 5 days
Fine, sharp-pointed scissors
Ringer's solution (warmed to around 38° C)
Fine, sharp-pointed forceps
Cotton wool
Culture dish
Paper towels
Small specimen jar
70% alcohol

Part E
One fertile egg that has been incubated 10, 14, 18, or 21 days
Scissors
Ringer's solution (warmed to about 38° C)
Specimen jar
Culture dishes
70% alcohol
Balances
Forceps
Paper towels
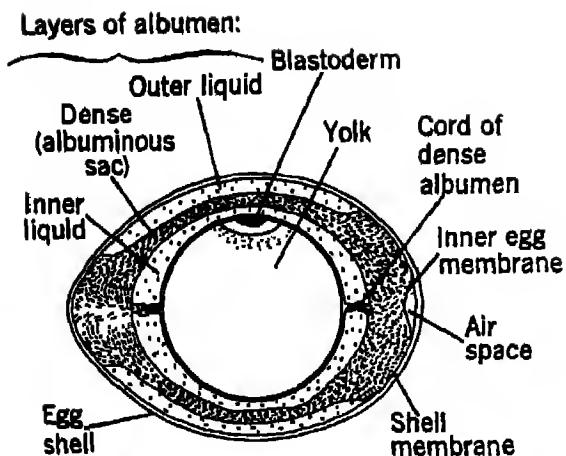
Figure 28-2-1 Internal structure of a chicken egg.

Part B:
The 48-hour Embryo

Open the incubated egg as instructed in Figure 28-2-2. An alternative method is to break the egg into a dish of warm saline (just as you break an egg into a frying pan). Locate the embryo and observe it. Then use the paper ring method shown in Figure 28-2-3 to remove the embryo from the yolk.

Place the Petri dish with floating embryo on the stage of a stereoscopic dissecting microscope and observe it under different powers. While you are observing the embryo, note carefully its stage of development. About all that you can see at this stage are the head and neck. Locate the two bulges on each side of the front part of the brain. Locate the paired blocks to the rear of the head. These paired blocks, called somites, will develop into the backbone and the muscles of the back. The brain and the nervous system develop from embryonic ectoderm, the somites from mesoderm. It is logical that bone and muscle develop from the same source as they are closely associated in the adult. Locate the heart. Is it beating? (1) Is there any sign of blood in the heart? (2)

Transfer the embryo from the Petri dish into a small bottle of alcohol and cap the bottle. Label the bottle 48-HOUR EMBRYO and date it.
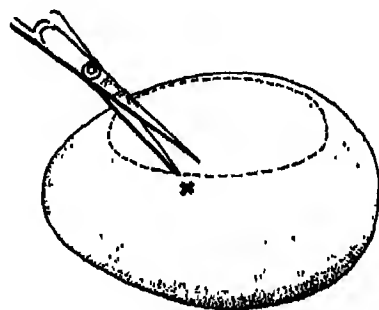
A Insert point of scissors carefully, barely through the shell at point corresponding to X.
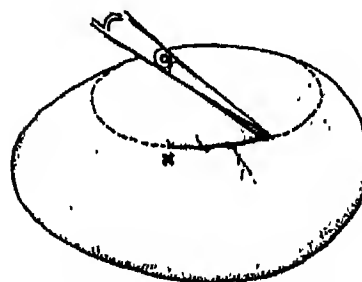
B Carefully and slowly clip the shell completely around the egg as shown.
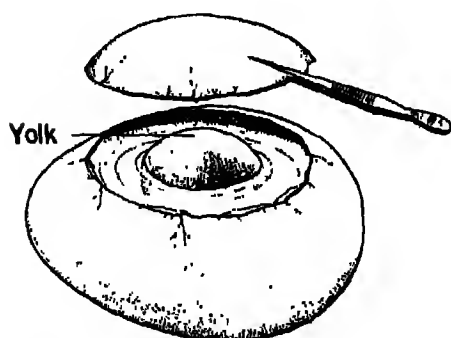
C With forceps carefully lift the loose piece of shell and discard it.
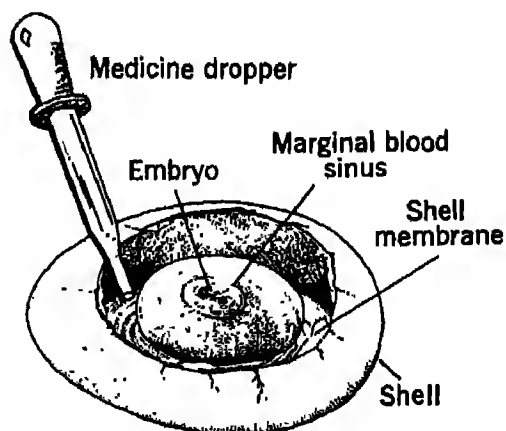
D Draw off albumen with medicine dropper until yolk surface is not covered.

Figure 28-2-2 Procedure for opening a 48-hour to 120-hour chicken egg.

Filter-paper ring

A Place filter-paper ring whose inside diameter is barely smaller than the diameter of the marginal blood sinus.

Petri dish with 10 ml physiological saline

C Lift the filter-paper ring with adhering membranes and embryo and transfer it to physiological saline.

B Grasp edge of paper ring and adhering membrane with forceps and clip the membrane completely around the paper ring.

Figure 28-2-3 Procedure for removing embryo from yolk.

Part C:
The 72-hour Embryo

Open, as shown in Figure 28-2-2, a fertile egg which has been incubated 72 hours. Remove the embryo from the yolk as shown in Figure 28-2-3.

While you are watching the embryo, notice carefully its stage of development. Observe the blood vessels around the embryo. To what area of the embryo are they connected? (3) Place the Petri dish and embryo on the stage of a microscope and observe it under low power. Locate the heart; then move the embryo until you can see part of the heart and some of the blood vessels. Notice the relationship between the pulsations of the heart and the surges (quick movements) of the blood. Can you distinguish the blood cells? (4) Observe the same structures you observed in the 48-hour embryo and describe the changes that have taken place. (5)

The circulatory system is derived from mesoderm. The heart is formed by the fusion of two straight tubes and later divides into four chambers. This type of heart is characteristic of birds and mammals.

Part D:
The 120-hour Embryo

CAUTION: *The yolk of the egg at this stage has become quite watery, and some of the delicate membrane will be close to the shell, so be very careful not to insert the scissors too far through the shell. Cut only with the tips.*

Open the egg as shown in Figure 28-2-2, or by cracking it into a dish of warm Ringer's solution. Locate the embryo, which should be on the upper surface of the yolk. Partially covering the embryo is a thin, delicate membrane with blood vessels at its surface. This membrane, called the **allantois**, will expand as the embryo develops until it completely lines the shell.

Next, look for the small chamber immediately surrounding the embryo. The membrane surrounding and forming this chamber is called the **amnion**. Probe the surface of the amnion, *gently*. With what does the amnion seem to be filled? (6) (This material is principally water from the egg white.)

You can see here that the embryo is actually developing in water, just as if it were a fish or frog.
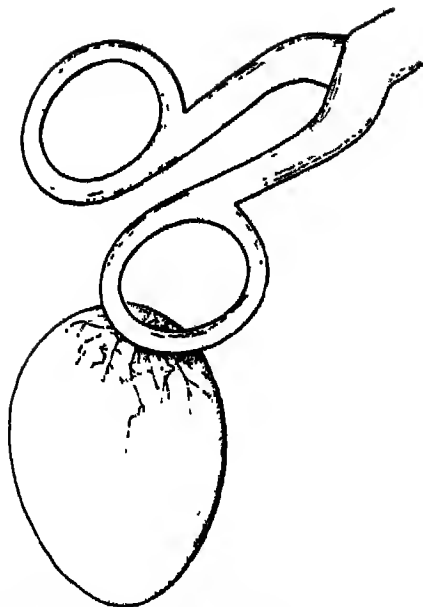
A Crack the large end of the egg with scissors or scalpel handle.
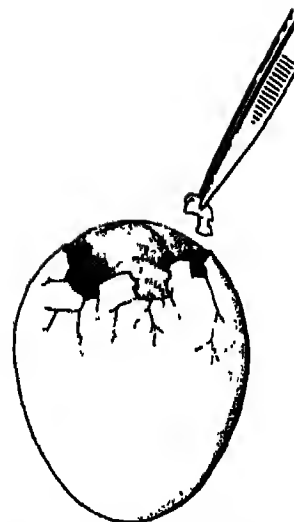
B Use forceps to pick away the shell. Avoid breaking shell membrane if possible.
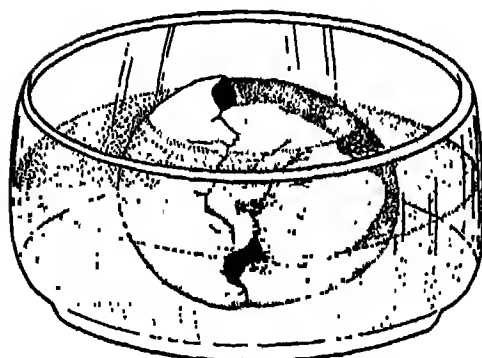
C After part of the shell has been removed, place the egg in finger bowl of physiological saline and pick off the remainder of the shell.
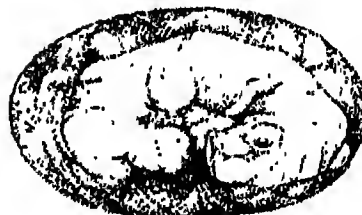
D All of shell removed.

Figure 28-2-4 Procedure for opening a chicken egg that has been incubated for 10, 14, 18, or 21 days.

Now open the amnion with forceps and scissors, being careful not to injure the embryo. Observe the embryo carefully—is there a neck separating the head from the trunk? (7) Does the embryo have a tail? (8) Look at the limb buds—would you describe them as wings or legs? (9) Look at the eyes and compare their size with the size of the embryo. These extra-large eyes are one of the distinct differences between bird embryos and other vertebrate embryos at the same stage. Adult bird eyes are also relatively larger than those of other land vertebrates. Compare the chick with a mammalian embryo at the same stage of development, if one is available.

Part E:
Older Embryos

You have already observed some of the earlier-stage chick embryos and their membranes. You will now open later-stage embryos and see how these structures change and new ones form as a chick develops. You will recall the tiny, almost transparent 48-hour embryo, the 72-hour embryo and the changes in it, and, finally, the 5-day embryo with its membranes clearly differentiated. Today, each team will open only one of the four stages to be observed. As soon as the members of your team have opened and observed the embryo, ex-

change it with a team which has opened a different stage. Since you will probably not make your observations in the order of their development, you will need to study your notes and rewrite them to start with the 10-day embryo and to end with the 21-day embryo.

In these stages the chorioallantoic membrane lines the shell; therefore, you will have to use a different procedure to open the egg. (See Figure 28-2-4)

As you carefully pick the shell away to expose the air space, you will see the outer shell membrane adhering to the shell and the inner shell membrane adhering to the contents of the egg.

Carefully remove the remainder of the shell as instructed. If you are skillful at this you can expose the whole chorioallantoic membrane which lines the shell. The chorioallantoic membrane encloses the embryo, amnion, and yolk sac and excludes only the albumen. Observe the blood vessels in this membrane and note their connection with the embryo through the chorioallantoic veins and arteries. Is the embryo still sealed in the fluid-filled amnion? (10) Figures 28-2-5, 28-2-6, and 28-2-7 will help you to identify these and the following structures.

Carefully remove the chorioallantoic membrane. Use the following questions to guide you in observing the membranes and embryo at each stage.

Is there a vascular sac surrounding the yolk? (11) Is there a yolk stalk that connects the yolk to the embryo? (12) What structures do you see that travel up through the stalk? (13)

What features make the embryo look like a bird? (14) How does it differ from other birds with which you are familiar? (15) Does it look specifically like a chicken, or just a bird in general? (16)

Have the eyelids developed yet? (17) Have the feathers developed yet? (18) How large are the legs? (19) Are there scales on the feet? (20) Are there claws on the toes? (21)

Has the chick poked its beak into the air space yet? (22) Is it now breathing air? (23) Is the chorioallantois sticky or somewhat dried? (24)

Locate the egg tooth (a hard, calcium mass on the tip of the upper beak) and the hatching muscle at the back of the neck. What is the apparent function of these two structures? (25) Has the yolk been drawn completely into the abdomen of the chick? (26)

Note: Since there are so many different observations to make on each embryo, it may
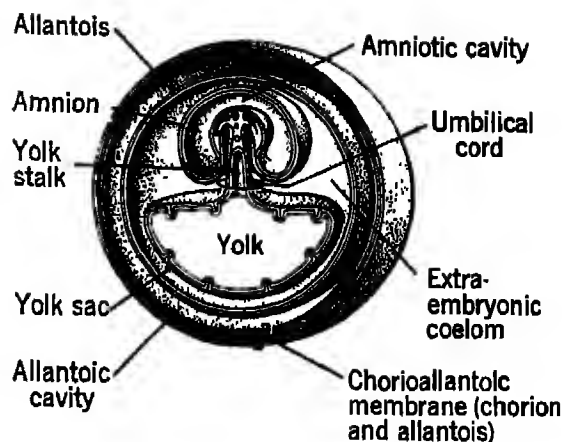
Figure 28-2-5 Transverse section of a late-stage chicken embryo.
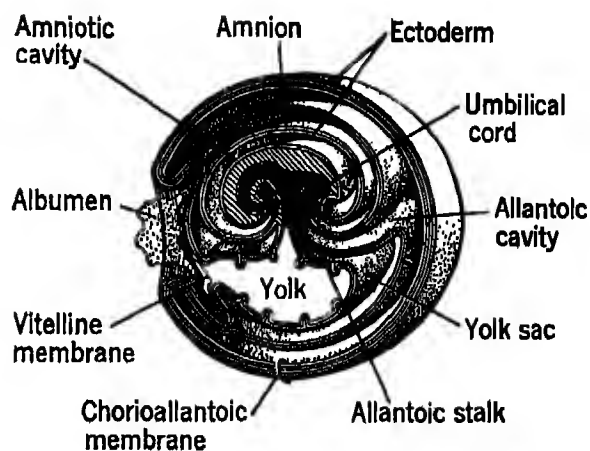
Figure 28-2-6 Sagittal section of a late-stage chicken embryo.
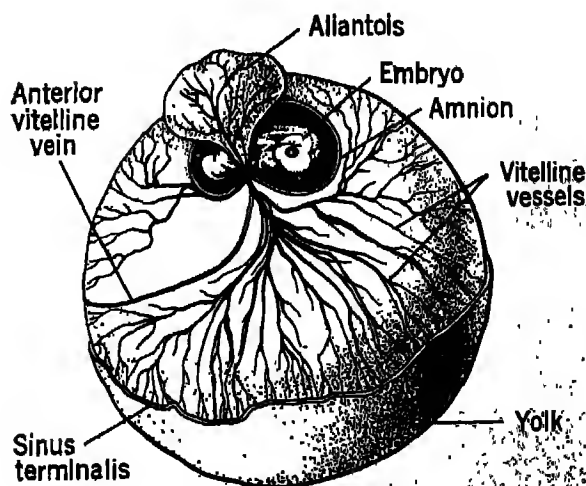
Figure 28-2-7 Six-day chicken embryo with yolk and membranes.

be wise for each member of a team to record data on different questions while in the laboratory.

As soon as you have observed the embryo which you opened, exchange embryos with another team. Observe carefully as many stages as time permits.

At the end of the laboratory period, carefully place the specimen in the jar of 70% alcohol to preserve the embryo you have used. Label the jar with both the date and the age of the embryo. At a later date you can examine the chick embryos that you did not have time to study today.

REGENERATION

Many living things have an amazing ability to replace, or regenerate, parts of their bodies that have been lost. Perhaps you have heard of the ability of some lizards to snap off tails when grasped by a predator, and then to develop a complete, new tail. Many amphibians can regenerate lost tails, legs, parts of the eye, or other organs. Among the invertebrates, regeneration is even more striking. Some of these lower animals are capable of replacing a head when it is cut off, or even regenerating a whole new body from a few hundred cells. The growth of whole plants from small stem cuttings can be considered a kind of regeneration.

Regeneration has been not only a sort of scientific parlor game but also a source of important information for developmental biologists. Perhaps you can see that regeneration represents in a mature animal a sort of return to embryonic conditions. Usually when a part is cut off, the wound heals and a small bud of undifferentiated cells appears at the cut surface. Gradually these cells divide, grow, and differentiate into a perfect replica of the missing part—just as the undifferentiated cells of the early embryo developed into a mature organism.

Many organisms are suitable for class study of regeneration. We could watch regeneration of heads in planarians, limbs in salamanders, tentacles in *Hydra*, tails in tadpoles, skin in humans. In general, however, the more complex and advanced an organism is, the less ability it has to regenerate lost or damaged parts. You may wish to observe several types of regeneration as a special project, since regeneration experiments often require extra time or special skill. We shall concentrate in this exercise on regeneration of two structures, the planarian body and the tadpole tail.

■ **The purpose of this exercise is to experiment with regeneration in an invertebrate (*Planaria*) and a vertebrate (tadpole) and to compare and contrast the process in both.**

MATERIALS

Part A
Planarians
Razor blade or fine scalpel
Microscope slide or large cork, for operating table
Small brush for picking up *Planaria*
Small Petri dishes or substitutes
Pond water or conditioned tap water

Part B
Tadpoles of various ages
Wide-mouthed pipette for transferring tadpoles
Fine-pointed scissors
Glass bowls
Petri dish or substitute
Narcotic solution (chloretone 1 : 2500 or MS222 1 : 2500)

PROCEDURE

Part A:
Planarian Regeneration

Type 1 Operation. Prepare three labeled dishes of water—one for anterior pieces, one for middle pieces, one for posterior pieces. Transfer a planarian from the stock dish to a slide or cork, using the brush to pick up the animal. Wait until the animal extends itself in crawling and then cut off the head as shown in Figure 29-1-1. Next, cut off the posterior end as shown. The pharynx is usually extruded and lost during the operation, and should be discarded. Place the pieces in the appropriate dishes. The experiment should be replicated several times, keeping all similar pieces in one dish.

Type 2 Operation. Follow the directions for a Type 1 operation, except make the cuts obliquely (at a slant) as in Figure 29-1-1.

Type 3 Operation. Using approximately the same technique as for Type 1, make a
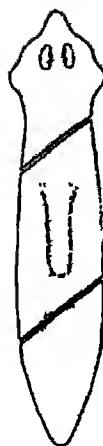
TYPE 1   TYPE 2   TYPE 3

Figure 29-1-1 Regeneration of Planaria.

single longitudinal cut, separating the right half of the animal from the left half, as shown in Figure 29-1-1.

Other Types of Operations. Certain more complex types of cuts can be made, and are used by students of regeneration to study special problems in development. If you believe you understand the problems being attacked with the types of operations described above, perhaps you can put your scientific ingenuity to work designing operations to produce the following results.

Type 4 Operation. Design an operation which will produce two heads on the anterior end, attached to a single body.

Type 5 Operation. Design an operation which will produce two heads on the same planarian, one head at the anterior end and one head at the posterior end.

Type 6 Operation. Design an operation which will produce two heads and two posterior ends in the same planarian.

Your teacher will discuss with you your proposed operations, and they might then be done as a special project.

No matter what types of operations you have performed, the care of operated pieces and the observations to be made are similar and are described below.

Transfer operated pieces to appropriately labeled dishes, cover the dishes, and keep in a dim light in a cool place. High temperatures are a chief cause of mortality. No food should be given to regenerating animals. Observe them daily and be careful to remove dead pieces. Change the water in any dish in which death of a part has occurred, as the products of decomposition are harmful to living pieces. Change all water every third day.

Make sketches showing the progress of regeneration. How soon does a regeneration bud appear? (1) How does the bud differ in appearance from the remainder of the piece? (2) In what ways does regeneration at the anterior end of a piece differ from regeneration at the posterior end of a piece? (3) Does a regenerated structure always grow perpendicular to the cut surface, so that a slanting cut produces a crooked planarian? (4) Check with other students if you did not do an operation pertinent to a given question. Is the speed of regeneration the same in cuts made at different places along the body? (5) What types of abnormalities appear in regenerated structures? (6) Does a middle piece seem to "know which end is anterior"—that is, does the anterior end ever form a tail? (7)

Part B:
Tadpole Regeneration

Transfer tadpoles to be operated upon into a Petri dish of narcotic solution, using the wide-mouthed pipette to make the transfer. When a given animal has become motionless, cut carefully across the tail membrane at the desired angle and transfer the animal to a culture dish of pond water. Feed the operated animals daily and change the water daily to remove unconsumed food. Make sketches just after the operation and daily thereafter.

How soon does the wound heal? (8) When does the first sign of regeneration appear? (9) When does the regenerated portion become pigmented? (10) Does the angle of cut affect the progress of regeneration? (11) What abnormalities appear in regenerated tails? (12)

CONTINUITY

Genetic Continuity

Thus far we have studied a variety of cells, microorganisms, plants, and animals. We have come to recognize those characteristics that are the same in all living organisms and, further, we have come to recognize those characteristics that distinguish major groups of living organisms one from another.

In this next section, we will deal with the processes whereby the characteristics of a particular type of organism are passed from generation to generation. It is customary for us to think of certain characteristics as being inherited. Our own general appearance, eye color and hair color, for example, readily may be recognized as traits which "run" in the family. The following sequence of exercises will demonstrate how characteristics are inherited from generation to generation. We will see "patterns" of inheritance and come to understand that these inherited patterns are a product of genetic continuity from generation to generation.

The experimental organisms we will use in laboratory will be either the fruit fly, *Drosophila*, or Indian corn (maize). In addition to performing genetic experiments in the laboratory with these organisms, we will have the opportunity to work with data concerned with biochemical aspects of genetics and with human inheritance.

These exercises, when completed, will give you an understanding of the role of genetic continuity in the process of inheritance.

DROSOPHILA TECHNIQUE

Before you begin to use fruit flies in genetics experiments it is necessary to learn to distinguish males from females, and to know about the life cycle of the fruit fly.

The common fruit fly, *Drosophila melanogaster*, is a mild household pest in almost every part of the world during the warmer seasons. It is found on rotting and fermenting fruit such as bananas, grapes, and plums. It is particularly common around fruit markets, warehouses, and garbage pails.

Drosophila is an excellent organism for studies in genetics because (a) it is easy to raise in the laboratory since it has simple food requirements and it takes up little space; (b) it can complete its life cycle in 10–12 days at room temperature; (c) it produces large numbers of offspring, (d) it may be anesthetized readily for examination and sorting; (e) it has many types of hereditary variations that can be recognized with low power magnification; (f) it is fairly hardy; (g) it has a small number of chromosomes (4 pairs) which are distinguishable and easy to study; (h) the cells of its salivary glands are large and contain giant banded chromosomes which serve to locate the positions of particular genes; and (i) much research and study of the genetics of *Drosophila* over the last 50 years have resulted in a wealth of reference literature and a knowledge of the location of hundreds of its genes.

Metamorphosis in insects involves even more dramatic changes than metamorphosis in frogs. In the description of the different stages in the development of *Drosophila* which follows, remember that the length of time each stage takes is determined by a number of factors. Of these, temperature is the most important. At 25° C the complete cycle will take about 10 days. As in the frog, the metamorphosis is controlled by hormones.

The Eggs. Adult female flies are capable of laying eggs after they are 2 days old, and from then on lay them more or less continuously until death. The eggs are very small, oval objects with two filaments on one end. They are usually laid in or close to the surface of the food, and with practice can be seen with the unaided eye. Examine them with the stereoscopic dissecting microscope. Sketch an egg as it lies on the surface of the medium.

The Larval Stage. The egg hatches into a larva after about a day. The larva molts twice as it increases in length. The first-stage larva sheds its cuticle (a noncellular layer covering the epidermis) and secretes under it a new and larger cuticle, permitting growth of the larva to the second stage. A similar process of shedding, known as **molting**, occurs between the second and third stages. The larva eats almost continuously, and its black mouth-parts can easily be seen moving back and forth in the medium even when the body of the larva is less distinct. Larvae channel through the medium while eating, and these channels, showing that the food has been "worked," offer a good indication of the successful growth of a given culture. In the last of the three larval stages the cells of the salivary glands contain giant chromosomes which may be readily seen, after proper staining, under the low-power objective.

The Pupal Stage. When it is ready to pupate, the mature larva usually climbs up the side of the bottle or onto the paper strip provided in the culture. The flies pupate inside their last larval covering, which becomes harder and darker, and changes its shape as it contracts. The later stages of metamorphosis in an adult fly can be observed through the pupal case. In particular, the eyes, the wings, and the legs are easy to see.

The Adult Stage. When the metamorphosis is complete, the adult flies emerge from the pupal case. They are fragile, light in color, and with wings not fully expanded. These flies darken in a few hours and take on the normal appearance of the adult fly. They live a month or more and then die. A female does not mate for about 10 hours after emerging from the pupa. Once she has mated, she stores a considerable quantity of sperms, and fertilizes her

eggs as she lays them. Hence to make a controlled mating it is necessary to use virgin females. These may be collected from a culture in which flies are emerging by removing *all* the adult flies, and then, after an interval of no more than 10 hours, collecting the freshly emerged flies. The females and males are separated and stored in separate containers until needed for making a cross.

■ The purpose of this exercise is to familiarize you with the life history of *Drosophila* and its metamorphosis, and to acquaint you with the techniques to be used in handling and culturing *Drosophila* as an experimental animal for use in genetic experiments.

MATERIALS

Vial of about 50 living fruit flies

Stereoscopic dissecting microscope, or a good hand lens

Etherizer

Ether

Very small tapered water-color brush, or a bent-tipped dissecting needle

Plate, such as a white plastic square of bathroom tile (10 cm, or 4 inches square), or a piece of porcelain glass (a piece of plain glass with a white card fastened underneath will also serve)

Rubber pad 7½ x 10 cm

Morgue (a jar with some old motor oil or xylene)

PROCEDURE

To examine the flies properly, they should be anesthetized with ether and then dumped out on the plate for observation through the dissecting microscope. Follow these steps in etherizing the flies. See Figure 30-1-1.

The instructor will demonstrate the proper way to "charge" your etherizer. When you put several drops of ether on the string around the neck of the funnel, place one finger beneath the neck. You will feel the ether when it reaches your finger. Do not put too much ether on the funnel. Ether vapor will anesthetize the flies, but contact with liquid ether will kill them. CAUTION: *Do not use ether near an open flame. Ether is highly inflammable!*

Place the funnel in the glass (Figure 30-1-1A)..

Gently but rapidly tap the bottom of the glass vial containing the flies on the rubber pad (or on your knee) to drive them momentarily to the bottom of the vial (Figure 30-1-1B).

Quickly remove the cotton plug from the vial, invert the open end and place it firmly into the funnel (Figure 30-1-1C).

Holding vial and etherizer firmly together, bump the bottom of the etherizer sharply once or twice on the rubber pad, on your hand, or on your thigh. This dislodges the remaining flies into the etherizer.

In the etherizer the flies will be overcome within a few seconds and will fall to the bottom. Watch them through the opening of the funnel or through the side of the glass, and as soon as the last fly stops moving, remove the funnel and dump the flies onto the porcelain plate for examination (Figure 30-1-1D).

Cotton plug

A   B   C   D   E
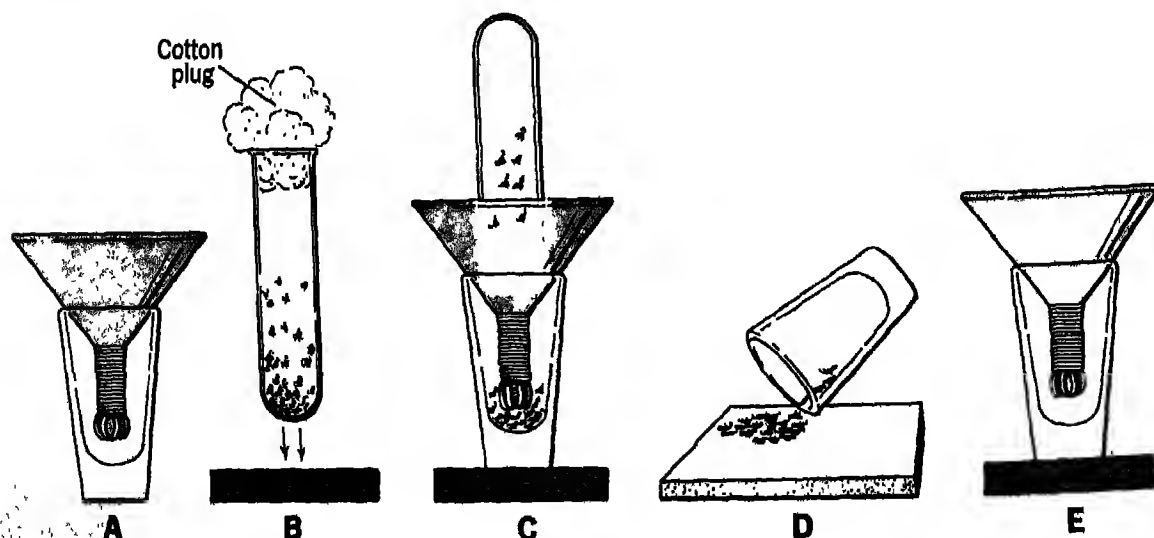
Figure 30-1-1 Anesthetizing Drosophila.

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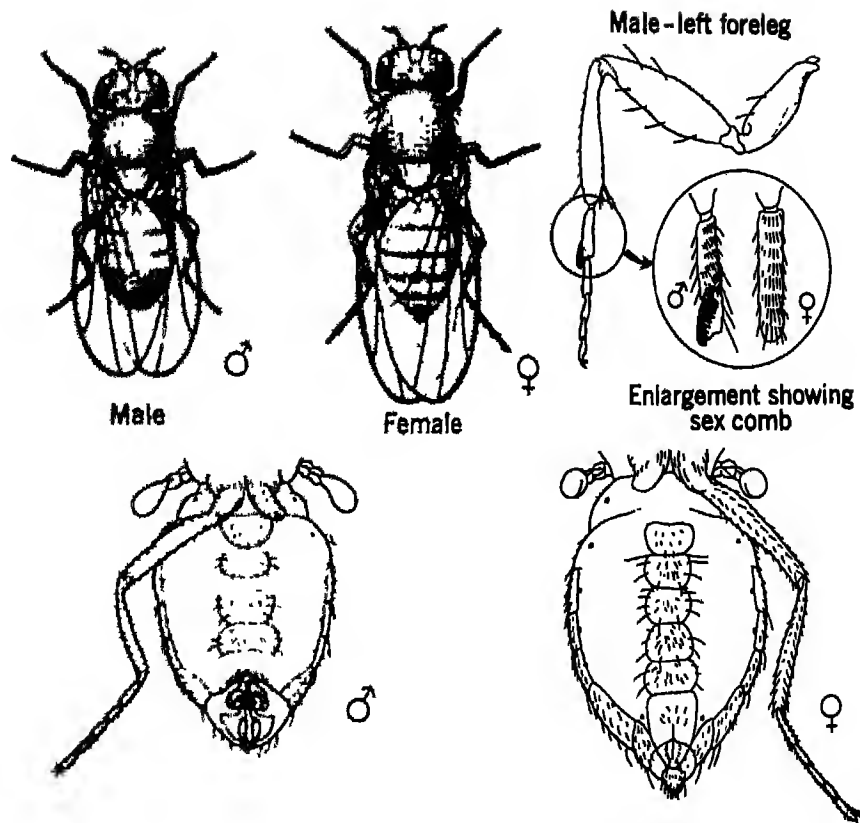
Figure 30-1-2 Male and female Drosophila.

SEX DIFFERENCES IN DROSOPHILA

Distinguishing Features	Comparisons	
	♂ (Male)	♀ (Female)
1. Relative total size (which is larger?)		
2. Kind of banding on abdomen		
3. Relative size of abdomen (which is larger?)		
4. Are sex combs present or absent?		
5. Shape of tip of abdomen		
6. Are external genitalia present or absent? Describe.		

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Immediately replace the funnel within the etherizer in order to prevent the fumes from escaping into the room (Figure 30-1-1E).

Do not over-etherize! Over-etherized flies will die. Over-etherized flies extend their wings vertically over their backs and then are difficult to handle and observe.

Since the flies may be injured easily, they should be moved around with a small brush or a bent-tipped dissecting needle while under observation. The stereoscopic dissecting microscope or hand lens is used in the examination. If the flies should begin to revive before the examination is completed they can be re-etherized. Etherized flies will recover in about 5 minutes.

Using the chart, Sex Differences in *Dro-* *sophila*, on page 215, look for the distinguishing parts. For each such feature, compare the male with the female and fill in the blanks under the columns ♂ (male) and ♀ (female). Figure 30-1-2 is included in this exercise to help you locate some of the more difficult parts, and also to substantiate previous findings. You will find it easier to compare the external genitalia by turning the flies onto their backs (ventral side up).

Be sure to return all living flies to the original vials as you complete your examination. *If the vials contain food, lay the vial on one side until the flies recover.* This prevents their sticking to the food in the bottom of the vial. These flies may be used by the next class. Dead flies should be placed in the morgue.

RANDOMNESS, CHANCE, AND PROBABILITY

Can chance itself be reduced to scientific laws? Does anything seem less likely? Yet it can, for a fact. Chance is a name we give to a situation when the causes that affect the outcome are so numerous, and individually so weak, that we can never hope to pick them out. But even when the causes can never be determined, there are certain mathematical aspects of the situation that we can study and reduce to statements of what to expect. These statements are what we call probabilities. Science deals very largely—some scientists say altogether—with probabilities, not with certainties. These probabilities explain what is most likely to happen when radioactive atoms are disintegrating, when the molecules of a hot gas are colliding, and when the genes are being passed from one generation to the next. It is of the utmost importance for every student of science to understand the nature of chance and the laws of probability.

Randomness is another term we apply to the element of chance in situations. "Choosing at random" is a common expression—one should not choose a wife or a president that way, but there are many situations where a random choice is the fair thing to make. It means a choice governed entirely by chance—hence, completely impartial.

Let us illustrate. A container is prepared holding 1000 marbles, 500 red and 500 black. They are thoroughly stirred. A blindfolded person reaches in and picks up a marble, the first one he happens to touch, and withdraws it. It is black. Now that is a random choice.

The marbles are thoroughly stirred, and then he reaches in and draws out another at random. Now the first choice involved equal probabilities of drawing red and black. Did the second one too?

No. At the time of the first draw there were equal numbers of red and black marbles in the containers, and they were distributed purely

by chance, so the probability of drawing either red or black was 50 : 50; that is, ½. But at the second draw, while there were still 500 red marbles in the container, there were only 499 black ones. The probability is ever so slightly higher that a red marble will be drawn this time; in fact, we can express the probability mathematically. It is in the ratio of 500 to 499, or 500 out of a total of 999, which is slightly greater than ½. So, although the second choice was still a random choice, its probability was different. However, had the black marble that was drawn been returned to the container before the stirring and the second draw, then the second draw would also have had a probability of red or black = ½, that is, 50 : 50.

Here is an important principle. If the first draw influences or biases the second draw, they are not independent of one another. Consider another example. In ancient wars it was sometimes a way of punishing or inflicting harsh discipline to decimate a company of soldiers or prisoners, that is, to kill every tenth man. Assume that the men are lined up in a row according to height, and the counting off starts from the highest man, on the left. Would this be a random selection of victims? Well, it might be, insofar as intelligence was concerned; but it would obviously not be random with respect to height. The shorter men, on the whole, would be selected. It would also not be random with respect to anything related to height, for example, weight. Evidently, any introduction of order into the items being chosen produces nonrandomness. If all the black marbles in the container were on top and the red ones on the bottom, how could the choice be random? That is why they must be thoroughly stirred, so the choice will be a matter of pure chance.

In heredity, we are concerned with the occurrence, every time an egg is fertilized or

the probability that a particular gene or chromosome will be passed on through the egg, or through the sperm, to the offspring. Now the genes and chromosomes, as you have already learned, are present in pairs in each individual, and segregate as they go into the gametes (eggs and sperms). There are two possible genes that the egg or sperm might obtain from each pair, but it actually receives only one of them. If the probability of getting either one is equal, this probability might be expressed as ½, like the probability of getting either heads or tails when you flip a penny. But one cannot examine the genes in a sperm or egg. One must wait until fertilization has occurred and a new individual has been produced, and some characteristic controlled by the alternative genes has had time to develop. Thus, we are faced with the probability that a certain gene will go into the egg, together with the probability that it will go into the sperm, together with the probability that these will combine at fertilization. Let us see how this will work out by using a model.

■ The purpose of this exercise is to demonstrate the principles of randomness, chance, and probability and their application to genetics and heredity.

MATERIALS (Parts A and B)

Two dice per team of students (different in color or size, if possible)
Dice cup (not essential)

PROCEDURE

Part A:
The Product Rule

We could perform our experiment by flipping pennies or by drawing red and black marbles out of a container with equal numbers of each. It is a bit quieter and easier to use dice. Take two dice and examine them. How many sides does each one possess? (1) Then what is the chance, on any throw, that a particular number will come up? (2) Of course, that would not correspond to the probability that one of two alternatives would be passed on in heredity; but how many even numbers are there on a die (sing. of dice)? (3) Then what is the probability that an even num-

ber—any even number—will come up? (4) What is the probability that an odd number will come up? (5) We can use the even numbers to represent the probability that a particular alternative gene or chromosome will go into the egg, when the female parent is heterozygous (*Aa*).

You are to work in pairs, first with one die only. Let one student in each pair roll the die, and the other record the result. Prepare a score sheet with two columns, headed ODD and EVEN. After every tenth throw, draw a line across the tally sheet, and total odds and evens. Continue to a total of 100 throws of the die, and find the grand total.

In every group of 10 throws, did you always get exactly ½ odd : ½ even? (6) Did you ever get exactly ½ odd : ½ even? (7) Is the total for 100 throws close to ½ odd : ½ even? (8) The difference between what you obtain and what you expect is known as the deviation. For example, in each lot of 10 throws, you expect by chance to get 5 odd : 5 even. Suppose you obtained, in one lot, 6 odd : 4 even. The deviation from expectation is then 1 for the odd group and 1 for the even group, or a total of 2, which is 20% of the 10 throws. Calculate the deviations for each of the 10 groups, as well as for the total of 100, expressing them as percentages. Are the deviations for the small groups of 10 larger or smaller, as a rule, than the deviation for the total of 100? (9) How does this demonstrate the importance of the *size of the sample* in all studies involving probabilities? (10)

All the teams in the class should now total their results, and the deviation should be calculated as a percentage. Does this additional increase in the size of the sample lead to a further reduction of the deviation from the expected ratio? (11) Is it a big reduction or a rather insignificant reduction? (12) Do the comparisons you have now made give you any idea as to how big an experiment of this kind should be? (13)

Each team should now throw two dice at once, letting one die represent the egg and the other the sperm. The combination that turns up will then represent the purely random probability of the odd and even eggs uniting with odd or even sperms in fertilization. To distinguish the die that represents the egg from that which represents the sperm, use dies of different color or size, or mark one die temporarily with an ink spot.

There will be four possible combinations of the odd and even numbers on the two dice.

Prepare a score sheet with four columns, headed respectively: BOTH EVEN; EGG EVEN, SPERM ODD; EGG ODD, SPERM EVEN; BOTH ODD. As before, let one student in each team roll the dice and one serve as recorder. Make a total of 40 throws.

What do you find to be the frequency of each of the combinations? Are they equal to each other? (14) If the results for the entire class are totaled, does that result in a closer fit to expectation? (15) What is the probability that any particular combination of the four will turn up, expressed as a fraction? (16)

What is the relation of the probability of one of these combinations to the probabilities of having an egg and sperm of the required types? For example, what is the relation of the probability of "Both even" to the probability of "Egg even" and the probability of "Sperm even"? Is it the sum, difference, or product of the probabilities? (17)

You have now demonstrated one of the most important principles of probability. It may be expressed in the following way. The probability of the combination of *independent* events is the product of their separate probabilities. (This is sometimes called the **Product Rule** of probability). Let us see. If that is true, we should be able to calculate the probabilities of the four classes in the preceding experiment by simple algebraic multiplication. We shall use E as a symbol for even and O as a symbol for odd.

Eggs: $(\frac{1}{2} E + \frac{1}{2} O)$
Sperms: $\times (\frac{1}{2} E + \frac{1}{2} O)$

What is the total product? (18)

It will be better for us to write EE instead of E^2 and OO instead of O^2 because in our actual combination of genes and chromosomes, these do not lose their identity and will separate again in the next generation. As for the combinations EO and OE, these are genetically the same. For the offspring it does not matter whether a particular gene has been inherited from the mother or the father, but only whether or not it is present. We can therefore add the two probabilities of OE and EO. The resulting ratio of the three classes corresponds to what ratio found by Mendel? (19) When is this ratio to be expected, that is, what must the genotypes of the two parents be like for this ratio to occur? (20)

Suppose that E were dominant over O, so that when the combination was EO, you could not distinguish it from EE—then what would

the ratio of even combinations to odd ones become? (21) To what genetic ratio does this correspond? (22)

Part B:
Boy or Girl?

A simple alternative in human inheritance is sex. Is the sex of an individual determined by chance? The previous example does not give us a ratio between two alternative types that is 50:50. Consider the Product Rule stated above, and then answer what kind of multiplication would yield a ratio of $\frac{1}{2}:\frac{1}{2}$. (23) In genetic terms, what would one parent have to be to produce this ratio? (24) What would the other parent have to be? (25) Later, in your study of genetics, you will discover how a particular pair of chromosomes brings this about.

To obtain data for this next part of the study, each student in the class is to bring in a record of his or her entire family of brothers and sisters. Include the sex of all of them, even if some are now dead; for example, two brothers plus one sister plus self, a total of four sibs (brothers and/or sisters). Obtain the total of males and females for all the families of all members of the class, and compare it with an expectation of $\frac{1}{2}:\frac{1}{2}$. Is there agreement? (26)

We can now apply what we have learned about the probabilities of combination of independent events to families of different sizes, to see whether sex really is independent from one birth to another in the same family. We can do this with dice to work out the probabilities. For families of two sibs, the experiment already performed with two dice that can be odd or even tells us what to expect. There should be twice as many families with a boy and a girl as there are families of two boys, or families of two girls. What is the expected ratio between families that have boys and girls and those that have only one sex represented? (27) Does the expectation based on the Product Rule hold good for the families of two children in your class? (28)

With three dice, we can determine the probabilities for families with three children; with four dice, for families of four children, and so on. If the Product Rule holds, we can obtain the result more quickly by multiplication. Multiply $\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2}$ which is the same as $(\frac{1}{2})^3$; or $\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} \times \frac{1}{2}$ which is the same as $(\frac{1}{2})^4$; etc. What is the probability of getting a family of three all of whom are boys? (29) Be careful

when you answer the next question: How often would you expect a family of three sibs to be composed of two girls and one boy? (30) You must remember that there are several probabilities that add up to this: girl, girl, boy; boy, girl, girl; girl, boy, girl. Those who have had algebra and have studied the expansion of the binominal will recognize that what we are calculating here is the expansion of the binomial expression $(a + b)$ to a power representing the number of sibs in the family, 2, 3, 4, or whatever. The binomial terms a and b are the chances of the simple alternative, male or female. Thus the expansion of

$$(a+b)^4 = a^4 + 4a^3b + 6a^2b^2 + 4ab^3 + b^4$$

tells us at once that out of a total of 16, which is the sum of the coefficients, one would expect four families to consist of three boys and one girl (a^3b) and six families to consist of two boys and two girls (a^2b^2).

What this study shows us is that we should actually expect, on the basis of chance and chance alone, to get some families composed entirely of boys or entirely of girls, even though the overall probability of being either male or female is $\frac{1}{2}$. We can test what actually happens to see whether or not it agrees with the expectation on the basis of chance alone. This was what Mendel did with his results. He found that the ratios he obtained in the F_2 generation (second filial generation) were simply those which would be expected if the probability were $\frac{1}{2}$ that an egg or pollen grain from the F_1 generation (offspring of a cross between two pure-breeding strains) would carry the recessive rather than the dominant allele, and if the probability of the union of eggs and sperms of different genotypes were random. Since chance alone would adequately explain his results, no other explanation was necessary.

INHERITANCE OF

ONE-FACTOR DIFFERENCES

Scholars from the days of the ancient Greeks to the middle (or even end) of the last century believed that traits in offspring were merely a blend of the parental traits, a sort of average. In 1858 Gregor Mendel started to test this belief by putting pollen from a tall strain of peas on the stigmas of a dwarf strain. The tall strain had produced nothing but tall plants and the dwarf strain had produced nothing but dwarf plants for many generations. Scientists of Mendel's day would have predicted that the offspring would be intermediate in height. We shall see if they were right and if not, why not.

Alternative A:
Cross Between
Two Strains of Drosophila

■ The purpose of Alternative A of this exercise is not to repeat Mendel's experiment but to discover the same principles by making crosses between two different pure-breeding strains of Drosophila.

MATERIALS

Alternative A

Basic equipment as in 30-1

Stock cultures of wild type (+) and mutant (M) (dumpy wings or sepia eye color)

F_1 cultures of wild X dumpy wings or sepia eye color (+ X M)

Culture vessels (vials, bottles, or paper vessels) containing fresh media

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PROCEDURE

So that you will have results to work with now instead of two weeks from now, your
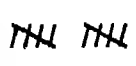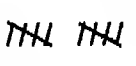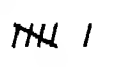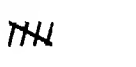
teacher has started some of the cultures several weeks before this period. You will make the matings to produce the second generation that you will then classify.

First examine some flies from stocks of the two parent types used in the cross, so that you know how they differ. One is a true-breeding normal or wild type and the other is a strain (also pure-breeding) that differs in a single respect from the wild type. This second strain, which may have, say, shorter wings than the wild type or a different eye color, is called a mutant strain, since a mutation is a heritable change and this strain has changed genetically from the wild form. Describe or make a sketch of the observable differences between the two types. The offspring of a cross between two pure-breeding strains, as you know, is called the first filial generation and is designated F_1. Classify each one of the F_1 offspring as male or female and as mutant, wild type, or intermediate, (having an appearance between the mutant and wild type) and enter it in the appropriate square of a six-fold tally sheet, arranged as shown on the following page.

The class will now be divided into three groups, to make three different crosses. Each team in the first group will take three F_1 males, and three F_1 females that have not been over-etherized, and place them together in a fresh culture vessel. The females need not be virgin. Can you explain why this is not required? (1)

Teams in the second group will each take three F_1 males and place them with three virgin wild-type females in a fresh culture vessel. Teams in the third group will each take three F_1 males and place them with three virgin mutant females in a fresh culture vessel. The virgin females will be supplied from the stock bottles, from which they are collected over an 8- to 10-hour period after all adult flies have been removed from the stock bottles. These

WILD TYPE × MUTANT

	♂	♀	
F₁			♂ = male
+	𝟙𝟙𝟙 𝟙𝟙𝟙 𝟙𝟙𝟙 𝟙 = 16	𝟙𝟙𝟙 𝟙𝟙𝟙 𝟙𝟙𝟙 = 15	♀ = female
			+ = wild type
Int			Int = intermediate
			M = mutant
M			

last two matings are called backcrosses: each is a cross of the F₁ back to one of the parent strains.

Seven or eight days after starting the matings, remove and discard the parents. The offspring of the next generation should start to emerge as adult flies on the tenth day. The offspring of the cross made by mating F₁ males and females together is the F₂ generation. The offspring of the two other crosses are called backcross progenies. Count the F₂ and backcross progenies for 8 to 10 days following the first day of their emergence. Do not count past the tenth day, or flies of a new generation may be included in the data. It is important not to discontinue backcross and F₂ counts after only 3 or 4 days because some mutant types may take a little longer for development than does the wild type. It has also been found that female flies appear in greater numbers during the first few days and males are more common during the last few of the 10 days allowed for one generation.

Make up a tally sheet to record the result of your classification of the F₂ or backcross progenies. This time a four-fold table will do. Why? (2)

All the teams performing the same cross are to compare and add together their data. Each student must also obtain the total results for each of the other two crosses done by the class and enter them in his notebook. Compare your own team's results with the total results from the same cross. Is there good agreement or poor agreement when both sets of results are put into percentages? (3)

Do you find that the F₁ flies have a blend of the parental traits? (4) How many different phenotypes were found in the F₁ generation? (5) (By phenotype we mean appearance.) We usually designate the phenotype by an adjective such as "brown" or an adjective and a noun, "brown eye." Does the mutant type appear in the offspring of either backcross? (6) If so, in which? (7) Is the mutant trait in these flies any different from that in the original mutant stock? (8) Compare the results of a mating of a wild-type fly from original wild (+) stock to the mutant type with the results of the mating of the F₁ wild type to the mutant stock. (9)

The F₁ wild type looks exactly like the parental wild type. What evidence do we have that they are different in a genetic sense? (10)

What do the above results indicate about the nature of the determiners of genetic traits? (11)

The differences in the offspring of *the backcross to the mutant type* must be traced to the F₁ parent (because the other parent comes from a pure-breeding stock). These differences must get into the offspring by way of gametes. How many kinds of gametes does the F₁ parent produce? (12) Compare the numbers of the two kinds of backcross offspring. Are they equal? (13) Of the tested gametes produced by the F₁ parent, what fraction (p) had the genetic determiner for wild type? (14) What fraction (q) had the genetic determiner for the mutant type? (15)

Since each gamete from the F₁ has either the wild-type gene or the mutant gene but not both, they are alternatives to each other. We call these alternative genes **alleles**. To show that two genes are alleles or alternates of each other, we use the same letter but distinguish one as follows—A, A' or A, a. For example, A and a are alleles and B and b are alleles, but A is not an allele of B.

If the F₁ flies look exactly like one of the parents (in this case the wild-type parent) we say the allele responsible for this trait is **dominant** and the allele which is unable to express itself in the F₁ is **recessive**. Is the mutant in your experiment dominant or recessive? (16)

Let us use m as the recessive allele and M as the dominant allele.

If M is on a chromosome from one parent of the F₁ and m is at a corresponding place on the homologous chromosome (which came

from the other parent), can you demonstrate why we obtained the particular values for p and q that we found above in Questions 14 and 15? (17)

Now p and q are the probabilities that an egg will carry the M allele or the m allele respectively. They are also the probabilities that a sperm will carry the M allele or the m allele. The possible combinations in F_2 through union of male with female gametes in fertilization may be shown in either of the following ways:

(1) by multiplying the two expressions

Eggs: $M + m$
Sperms: $\times M + m$
$$\overline{MM + Mm}$$
$$\underline{\qquad Mm + mm}$$
$$MM + 2\,Mm + mm$$
Ratio 1 : 2 : 1

or (2) graphically by a square:

EGGS

Fractions $\frac{1}{4}$ MM
$\frac{1}{2}$ Mm
$\frac{1}{4}$ mm

Both of these methods assume two things: (a) that p and q, the probabilities of M and m being passed on, are equal; that is, that each is $\frac{1}{2}$; and (b) that fertilization is *random*.

What would result if p were twice as great as q? (18) What would occur if M eggs were always, or nearly always, fertilized by M sperms, and m eggs by m sperms; that is, if fertilization was not at random but was preferential? (19)

Now examine the class's F_2 data. Do they agree with the expected ratio given above? (20) Test this by using the chi-square method (see Appendix) to compare your observed F_2 results with those predicted. Use the above chart for computation. Do your results differ significantly from the expectation? (21)

If you find that the F_2 results are not significantly different from the expected ratio, what can you conclude about the values of p and q in this cross? (22) What have you learned

about the nature of fertilization? (23)

Work out the possible combinations of the kinds of male and female gametes in the two backcrosses. (24) What does this lead you to expect in the backcross to the mutant strain? (25) For the backcross that yields two classes of offspring, test the significance of the difference between the results of your class and the expected ratio. Use the chi-square method and record your answer. (26)

Write in your notebook a clear statement for each of the Mendelian principles illustrated in this exercise. (27)

Alternative B:
Cross Between Two Strains
of Maize (Indian Corn)

■ The purpose of Alternative B is to discover Mendel's principles with crosses between two different pure-breeding strains of maize.

MATERIALS

Alternative B
Ears of corn segregated for color of kernels
(F₂ ears from original cross of purple X
nonpurple; backcross ears, F₁ X nonpurple
parent strain)
Straight pins

The Mendelian principles are known to have wide application among many kinds of organisms. Inheritance in many plants, especially corn, is as well known to biologists as inheritance in fruit flies. Studying inheritance in corn grains has certain basic advantages. One is the speed with which the observations can be made because the grains are held in place on the ear of corn and this makes counting of individuals easy.

Each corn grain on the ear you will use represents a separate offspring of a cross between the female parent (of which the ear and the corncob are part) and the male parent, which supplied the pollen grains that fertilized the ovules which then grew into the corn grains (seeds). The pollen grains are produced in the tassel of the corn plant. Normally it is the wind which carries the pollen grains to the silks of the ears of corn. The pollen tubes grow out from the pollen grains and down the silks, and the male reproductive cell eventually reaches the egg cell and unites with it.

The fertilized egg cell then grows into the embryo of the seed, and the endosperm is formed in a similar way (see Chapter 17 of the textbook). The colored layer of the seed that you will see is a part of the endosperm. The seed therefore represents the offspring, or progeny, of the mother plant on which the ear is borne.

The endosperm or the embryo of the corn grain can exhibit differences of an inherited nature, such as the color of the outer layer of the endosperm (purple or nonpurple) or the character of the surface (smooth or shriveled). Our problem is to discover how such a difference is inherited and how the mode of inheritance compares with that of other organisms, such as Mendel's peas or Morgan's fruit flies.

In producing these ears, the plant breeder selected parents that were pure-breeding: that is, one whose offspring always form purple grains and one with offspring forming only cream-colored, nonpurple grains. The pollen from one of these strains was placed on the silks of ears of the other strain, and seeds were then produced belonging to the first filial generation (F_1) of the cross. These F_1 seeds were planted, grew into mature maize plants, tasseled and put forth their silks. Then the plant breeder made two kinds of crosses, and these are the ears you will examine. One cross was simply to place pollen from each F_1 plant on silks of the same plant. (The silks are protected from wind-pollination before and after the desired cross is made by having a small bag put over them.) The second kind of cross takes pollen from tassels of an F_1 plant and places it on silks of the original nonpurple strain. We call the progeny of the first kind of cross the F_2 generation; and we call the progeny of the second kind of cross a backcross generation. Half of the teams in the class will receive F_2 ears to examine; the others will get the backcross ears to classify.

You may also have a few of the F_1 ears available for study, but since this may not be the case, it is fair to tell you that all the F_1 seeds are purple-colored. When two alternative types are crossed, such as purple and nonpurple seeds, the offspring are uniform in type if the parent strains are pure. But there is no way to tell in advance of a test whether the character of the F_1 will be always purple, always nonpurple, or always intermediate. If it turns out that all the F_1 grains are purple, the purple characteristic is said to be dominant over the alternative nonpurple characteristic, which is said to be recessive.

PROCEDURE

Each student team should receive an ear of corn. One student may then classify the kernels, while the other acts as a recorder. You will not be told whether it is an F_2 ear or a backcross ear. This is something you must find out. Record on a data sheet with two columns, one for each color, the number of kernels of each type. Tally each in the appropriate column. The student classifying the kernels places a pin in the ear at the end of the row of kernels where he starts counting. Then he calls off the characters of each kernel in each row until he returns to the starting point marked by the pin. As the characters are called out, the student doing the tallying records each in its proper place on the data sheet.

CAUTION: Be careful when handling the ears or the kernels will become loose and drop out. *Do not pick the kernels from the ears!*

Total the number of purple and nonpurple seeds recorded on your ear of corn. Calculate the percentage of purple seeds. (1) Calculate the percentage of nonpurple seeds. (2) Are the two percentages close to any ratio of small whole numbers? If so, what? (3)

Look carefully at the nonpurple seeds. Remember that they came from a cross in which at least one parent was purple. Is there any trace of purple color in these F_2 and backcross seeds? (4) What can you conclude about the effect upon the gene producing a recessive trait of having been present for a whole generation in company with a dominant gene that masked its presence? (5)

The F_1 purple seeds look exactly like the original purple seeds. What evidence do you have that they are different genetically? (6)

What do the above results indicate about the nature of the determiners of genetic traits? (7)

The differences in the seeds of the backcross progeny must be traced to the F_1 parent (because the other parent comes from the pure nonpurple strain). These differences must get into the offspring by way of the gametes. Since each gamete from the F_1 has either the gene for purple or a gene for nonpurple and never carries both, the purple-carrying gametes and the nonpurple gametes ought to be formed in equal numbers. Then, since only the nonpurple kind of gamete is supplied by the backcross parent, what is to be expected in the progeny? (8) Do any of the ears examined manifest this ratio? (9) What fraction (p) of tested gametes produced by the F_1 parent carried a gene for purple? (10) What fraction (q) had the gene for nonpurple? (11) Compare

the numbers of the two kinds of seeds with the numbers to be expected in the same total if the two kinds of gametes produced by the F_1 parent were equally common; and test the significance of the difference by means of the chi-square method (see Appendix). Use the chart for computation and record your results and conclusion. (12)

If there is good agreement, what does this prove with respect to the genes for purple and nonpurple seed color in the F_1? (13) Alternative genes that always segregate from one another are called alleles. To show that two genes are alleles of each other, we use the same letter: A, A' or A, a. For example, A and a are alleles and B and b are alleles, but A is not an allele of B. We can use A and a for the alleles in our crosses, for purple (anthocyanin pigment) and nonpurple (no anthocyanin pigment) respectively.

If A is on a chromosome from one parent of the F_1 and a is at a corresponding place on the homologous chromosome (from the other parent), can you demonstrate why we obtained the particular values for p and q that we found in Questions 10 and 11? (14)

Now p and q are the probabilities that an egg cell will carry the A allele or the a allele respectively. They are also the probabilities that a pollen grain will carry the A allele or the a allele. The possible combinations in F_2 through union of male with female gametes in fertilization may be shown in either of the following ways:

(1) by multiplying the two expressions

$$\text{Eggs:} \quad A + a$$
$$\text{Pollen grains:} \times \underline{A + a}$$
$$\overline{AA + Aa}$$
$$\underline{\qquad\qquad Aa + aa}$$
$$\overline{AA + 2Aa + aa}$$
$$\text{Ratio:} \quad 1 \;\; : \;\; 2 \;\; : \;\; 1$$

or (2) graphically by a square

EGGS

	A	a
A	AA	Aa
a	Aa	aa

POLLEN GRAINS

Fractions

$\frac{1}{4}\,AA$

$\frac{1}{2}\,Aa$

$\frac{1}{4}\,aa$

Both of these methods assume two things: (a) that p and q, the probabilities of A and a being passed on, are equal; that is, that each is $\frac{1}{2}$; and (b) that fertilization is *random*.

What would result if p were twice as great as q? (15) What would occur if A eggs were always, or nearly always, fertilized by A pollen grains? and a eggs by a pollen grains? that is, if fertilization was not random but preferential? (16)

Now examine the class's F_2 data. Do they agree with the expected ratio given above? (17) Test this by using the chi-square method (given in the Appendix) and record your result. (18)

If you find that the F_2 results are not significantly different from the expected ratio, what can you conclude about the values of p and q in this cross? (19) What have you learned about the nature of fertilization? (20)

Write in your notebook a clear statement for each of the Mendelian principles illustrated in this exercise. (21)

INDEPENDENT INHERITANCE

What will happen when two mutant types not governed by genes at the same location, or even on the same chromosomes, are crossed? This is called a dihybrid cross. Suppose, for example, a fruit fly with dumpy wings but normal red eye color is crossed with one that has normal wings but a dark-brown eye color, known as sepia. Will the progeny be the same in the first and second generations as those resulting when a fruit fly with both dumpy wings and sepia eye color is crossed with a type having both normal wings and normal eye color? Will the offspring of different types be in the same proportions?

Alternative A:
Using Drosophila

■ The purpose of Alternative A is to determine by means of a dihybrid cross how characteristics governed by separate genes on different chromosomes are inherited.

MATERIALS

Alternative A
Virgin females of one type and males of the opposite type—for one of the following crosses:
  Cross a. Dumpy-winged (red, or normal-eyed) virgin females with sepia-eyed (long, or normal-winged) males
  Cross b. Sepia-eyed (long, or normal-winged) virgin females with dumpy-winged (red, or normal-eyed) males
  Cross c. Dumpy, sepia virgin females with wild-type (long-winged, red-eyed) males
  Cross d. Wild-type virgin females with dumpy, sepia males
Two fresh culture vials or bottles for the initial cross; two more for the cross of F₁ flies
Basic materials as in 30-1

PROCEDURE

Mate the virgin females of your assigned cross with the appropriate type of males, placing 2 females and 3 males in a vial or bottle. After 7 days, if there is evidence of larvae in the culture, remove the parent flies and dispose of them (morgue). When the F_1 generation emerges, in 10 to 12 days, examine at least 50 flies for their wing length and eye color. Classify each fly for *both* characteristics. How many types do you find? (1) Are dumpy and sepia respectively dominant or recessive? (2) Record this information on a data sheet. How would you write the full genotype of an F_1 fly? (3)

Mate 3 or 4 pairs of the F_1 flies by placing them in fresh culture vials or bottles. The females need not be virgin. Why not? (4) After 7 days, remove these F_1 parents of the F_2 generation from the cultures and dispose of them.

When the F_2 flies emerge, 10 to 12 days after starting the second set of cultures, examine at least 250 flies and classify them into types by sorting them into piles. How many combinations of wing length and eye color can you find? (5) How many can be expected if all possible combinations occur? (6) What is the ratio of all the normal-winged individuals to all the dumpy-winged individuals? (7) What is the ratio of all the red-eyed individuals to all the sepia-eyed individuals? (8) Test each of these ratios by the chi-square method (given in Appendix III). Do these ratios differ significantly from the ¾ : ¼ ratio that you would expect if each abnormal trait depends on a single mutant gene? (9)

If the 2 pairs of traits are inherited quite independently, then the combinations of the 2 wing types and the 2 eye colors should occur with frequencies equal to the products of the frequencies of the single types; for example, the frequency of dumpy-winged, sepia-eyed flies should be the product of the fraction of dumpy-winged flies in the total multiplied by the fraction of the sepia-eyed flies in the

total. Therefore, if each pair of traits segregates into a $3/4 : 1/4$ ratio, the product would be as follows:

$3/4$ nondumpy + $1/4$ dumpy
× $3/4$ nonsepia + $1/4$ sepia

$9/16$ +	$3/16$ +	$3/16$ +	$1/16$
nondumpy	dumpy	nondumpy	dumpy
nonsepia	nonsepia	sepia	sepia

(Instead of using fractions, you may equally well use the decimals 0.75 and 0.25 to calculate this product.)

The same product may be obtained by combining eggs and sperms. Let d be the dumpy gene and D its wild-type allele. Let s be the sepia gene and S its wild-type (red) allele. If, firstly, the female is $DdSs$; and if each gamete must get either D or d, and S or s; and, secondly, if the two pairs of alleles are independent of each other (that is, they are on two pairs of chromosomes), then the expression for the eggs is ($1/4$ DS + $1/4$ Ds + $1/4$ dS + $1/4$ ds).

For the same reasons, the expression for the sperms is ($1/4$ DS + $1/4$ Ds + $1/4$ dS + $1/4$ ds). To obtain the offspring we combine eggs and sperms at random:

($1/4$ DS + $1/4$ Ds + $1/4$ dS + $1/4$ ds)
× ($1/4$ DS + $1/4$ Ds + $1/4$ dS + $1/4$ ds)

$1/16$ $DDSS$	
$2/16$ $DDSs$	$9/16$ nondumpy nonsepia
$1/16$ $DDss$	
$2/16$ $DdSS$	
$4/16$ $DdSs$	$3/16$ nondumpy sepia
$2/16$ $Ddss$	
$1/16$ $ddSS$	$3/16$ dumpy nonsepia
$2/16$ $ddSs$	
$1/16$ $ddss$	$1/16$ dumpy sepia

Check this product by using the checkerboard method, putting the 4 kinds of eggs along one side and the 4 kinds of sperms along an adjacent side (one at a right angle to the first); then enter the correct combination of egg and sperm in each square and add them up. How many genotypes are present in the F_2? (10)

Did you find all 4 phenotypes in the F_2? (11) Was the wild type most common? (12) Was the double-mutant type the rarest? (13) Were the single-mutant types (dumpy without sepia and sepia without dumpy) about equally common? (14) Is the total distribution you obtained significantly different from $9/16 : 3/16 : 3/16 : 1/16$ when tested by the chi-square method? (15)

Compare your results with those obtained by other teams doing the same cross as yours. Pool the data. Now compare the class results from crosses a, b, c, and d. Are they alike or different? (16)

Two separate pairs of genes would be expected to segregate and recombine quite independently if 1 pair of genes were on 1 pair of chromosomes and the other pair of genes were on a different pair of chromosomes. What Mendelian law does this exercise illustrate? State it carefully, in full. (17)

Alternative B:
Using Maize Kernels

Although plant material has certain advantages in studies in genetics, it is usually slow-growing, and it is impossible, in the time allotted to the laboratory, to make the crosses and wait for the arrival of two generations to complete our observations. This could take one or two years when using corn plants.

To obtain the ears of corn you will use, a mating was made between pure-breeding plants, one whose offspring always form purple, smooth kernels and one whose offspring form nonpurple (cream-colored), wrinkled (shrunken) grains. We are not going to tell you what the offspring (F_1 generation kernels) resulting from the mating of parents described above were like. You should be able to determine this from subsequent observations of the F_2 kernels.

■ The purpose of this exercise is to determine, by means of a dihybrid cross, how characteristics governed by separate genes on different chromosomes are inherited.

> **MATERIALS**
>
> *Alternative B*
> Ears of maize (corn) segregating for purple color and shrunken kernels
> Straight pins

PROCEDURE

The procedure is the same one used in Exercise 30-3 (Alternative B). Two students work together, one acting as the tally clerk, the other classifying the corn grains. Remember to place a pin in the ear at the point where you

started counting. CAUTION: Handle the ears with care so the kernels will not come loose. *Do not pick the kernels from the ear!*

How many kinds (phenotypes) of kernels are there in the F_2? (1) Which type is most common? (2) Which is the rarest? (3) Are the types purple shrunken and nonpurple smooth about equally common? (4) From your observations you should be able to tell which genes are dominant and which are recessive. Using symbols for the pairs of alleles (A or a for the seed color; S or s for the texture), and letting the capital letter stand for the dominant allele and the small letter for the recessive allele in each pair, write down the genotypes of the F_1 generation that produced the ear that you are examining. (5) From these F_1 genotypes, can you tell what their two parents were like, supposing each of them to have been a pure strain? Explain. (6)

In many plants—and corn is no exception—it is possible to obtain an F_2 generation by selfing: the pollen grains (male) are placed on the silks of the ear (female) on *that same plant.* This is another advantage that plant material has over most animal material in genetic studies. We assume, when selfing a plant, that the types of sperms and eggs and the relative proportions of the types will be the same genetically. Why is this reasonable? (7) If the types of eggs and sperms of the selfed F_1 plant are indeed identical in their genetic make-up, we can predict the classes, or types, of offspring that will be produced by the F_1 plants.
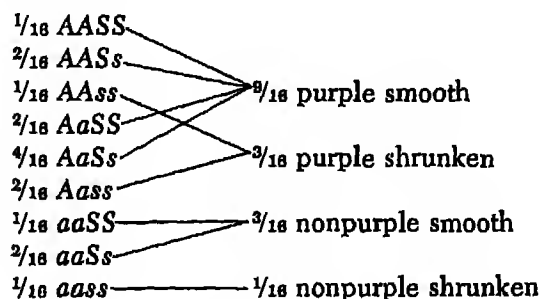
If the 2 pairs of traits are inherited quite independently, then the combinations of the 2 seed colors and the 2 seed textures should occur with frequencies equal to the products of the frequencies of the single types; for example, the frequency of nonpurple shrunken seeds should be the product of the fraction of nonpurple seeds in the total multiplied by the fraction of the shrunken seeds in the total. Thus, if each pair of traits segregates into a $3/4$:$1/4$ ratio, the product would be as follows:

$$\frac{3}{4} \text{ purple} + \frac{1}{4} \text{ nonpurple}$$
$$\times \quad \frac{3}{4} \text{ smooth} + \frac{1}{4} \text{ shrunken}$$

$9/16$ +	$3/16$ +	$3/16$ +	$1/16$
purple smooth	nonpurple smooth	purple shrunken	nonpurple shrunken

(Instead of using fractions, you may use the decimal equivalents 0.75 and 0.25 to calculate this product.)

The same product may be obtained by combining eggs and sperms. Let a be the nonpurple gene and A its purple allele. Let s be the shrunken gene and S its smooth allele. The expression for eggs is ($1/4$ AS + $1/4$ As + $1/4$ aS + $1/4$ as). This is true because, firstly, as the F_1 plant is $AaSs$, each gamete (egg) must get either A or a, and S or s; and, secondly, the two pairs of alleles are independent of each other (that is, they are on two pairs of chromosomes).

For the same reasons the expression for sperms is ($1/4$ AS + $1/4$ As + $1/4$ aS + $1/4$ as). To obtain the offspring we combine eggs and sperms at random:

$1/16$ $AASS$
$2/16$ $AASs$
$1/16$ $AAss$
$2/16$ $AaSS$ $9/16$ purple smooth
$4/16$ $AaSs$
$2/16$ $Aass$ $3/16$ purple shrunken
$1/16$ $aaSS$
$2/16$ $aaSs$ $3/16$ nonpurple smooth
$1/16$ $aass$ —————— $1/16$ nonpurple shrunken

Check this product by using the checkerboard method, putting the 4 kinds of eggs along one side and 4 kinds of sperms along an adjacent side (a side at a right angle to the first); then enter the correct combination of egg and sperm in each square; and add all those that have the same genotype. How many genotypes are to be expected in the F_2? (8)

Add together all kernels that have an identical phenotype. Is the total distribution you obtain significantly different from $9/16$: $3/16$: $3/16$: $1/16$ when tested by the chi-square method? (9)

Compare your results with those obtained by the other teams in the class. Pool the data. Now test the significance of the difference between the entire class's results and a 9 : 3 : 3 : 1 ratio by means of the chi-square test. Is the agreement better or poorer than when only the data from one (your own) team are tested? (10) What does this comparison indicate regarding the importance of the size of a sample in a genetic or other scientific experiment? (11)

Two separate pairs of genes would be expected to segregate and recombine quite independently if one pair of genes were on one pair of chromosomes and the other pair of genes were on a different pair of chromosomes. What Mendelian law does this exercise illustrate? State it carefully, in full. (12)

HEREDITY AND ENVIRONMENT

As you have observed in previous exercises of this section, hereditary materials (genes) are the determiners of the characteristics of living things. Further, you have seen that these genetic materials are inherited according to certain patterns that can be predicted. With this amount of understanding we are ready to discover the way or ways in which environment can modify the development of the inherited potentialities.

In this particular exercise we will start with tobacco seeds that were produced from crossing parents whose genotypes carried a recessive gene for albinism (which inhibits chlorophyll formation) and a dominant gene for normal chlorophyll production. Knowing this, we can apply what we have learned about the segregation and recombination of alleles and predict the kinds and numbers of kinds of plants we will obtain when we germinate the tobacco seed. Prepare a diagram showing the genotypes of the parents, the gametes they form, and expected genotypes and phenotypes of their offspring. (1) We will compare these theoretical results with results obtained from the experiment and attempt to determine the relative roles of heredity and environment on the basis of our results.

■ The purpose of this exercise is to discover a way in which environment can modify the development of the inherited genetic potential of an organism.

MATERIALS

Four culture dishes (Petri dishes or paper ice cream containers with covers)

Filter- or blotting-paper circles to fit bottom of dishes (for tobacco seeds); or vermiculite, peat moss, or sand (for corn seeds)

Lightproof covers for culture dishes

Tobacco or maize (corn) seeds from parents heterozygous for albinism

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PROCEDURE

Sprinkle about 30 tobacco seeds evenly over the moistened paper in each of the four culture dishes; or plant corn seeds in moist vermiculite. No seed should be closer than twice its own length to another seed. Replace the covers and place the dishes in a well-lighted place but not in direct sunlight. Cover two dishes with a cardboard box or some other lightproof covering. Let the seeds germinate for 8 days, watering them about every other day with a few *drops* of water if they tend to get dry. On the eighth day, you may begin to make entries of results in the tables that your teacher will give you. Some of the seeds give rise to albino plants, which lack color. Other seedlings show the usual green color as they develop. Count the number of each kind of plant occurring under each set of conditions and record in the appropriate place in the table.

When most of the plants have started to develop, remove the coverings permanently from the two covered dishes and continue to record the appearance of each seedling for two more days, or until nearly all the seeds have germinated.

Among the seedlings that were kept in the light, how might you account for the differences observed? (2) Would the differences be attributable to heredity or to environment? (3) Are the observed numbers of green and albino plants close to the ratio you expected? (4)

Among the seedlings that were kept covered, how do the percentages of albino and green compare with the percentages found among the uncovered seedlings? (5) Is this difference to be attributed to one or the other of the factors indicated in the title of this exercise? (6) What do you think is responsible for the appearance of the covered seedlings? (7)

Among the seedlings first covered and then uncovered, how do the percentages of green and albino seedlings compare after the coverings have been permanently removed with the percentages previously recorded when the

plants were still under cover? (8) How might you explain what happened after the coverings were permanently removed? (9) State what you think is responsible for the appearance of differences in the seedlings that were first covered and later uncovered. (10) What has been shown about the modifying effects of one particular environmental factor upon seedlings possessing definite hereditary possibilities? (11) What is the relative importance of environment and heredity in the color of tobacco (or maize) seedlings? (12)

In all previous experiments, especially those in which fruit-fly cultures were used, environmental conditions have been optimum for development and have remained constant. After completion of this experiment it must be obvious why we maintain constant environmental conditions when we are studying the effects of inherited characteristics in living organisms. Of course, controlled conditions are also essential for other biological experiments in order to avoid unwanted environmental effects.

LINKAGE AND CROSSING OVER

Suppose that 2 mutant genes lie in the same chromosome at different locations. One would hardly suppose that they would be inherited independently. It would seem as though they would have to be transmitted together to the same offspring. Or suppose the same 2 mutant genes had arisen in different lines of ancestry that then had mated, so that a hybrid offspring was produced with 1 mutant gene in a certain chromosome and the other mutant gene in the homologous chromosome (the other chromosome of the same pair). In meiosis, as you have learned, homologous chromosomes pair together, then segregate; so a gamete receives not both homologous chromosomes, but only 1 of them. In this case, one would conclude, the 2 mutant genes would be regularly transmitted to *different* offspring of the hybrid individual. Will it always work out this way?

■ The purpose of this exercise is to determine the patterns of inheritance when genes are linked together on the same chromosome and to determine the possible recombinations which arise from crossing over.

Alternative A:
Linkage and Crossing
Over in Drosophila

PROCEDURE

Team 1: Mate virgin double mutant females carrying sepia eye color and spineless bristles (or brown eye color and vestigial wings) with wild-type males.

Team 2: Mate virgin wild-type females with the double mutant males.

Team 3: Mate virgin sepia nonspineless females with nonsepia spineless males.

Team 4: Mate virgin nonsepia spineless females with sepia nonspineless males. Use 2 or 3 pairs for a vial culture, about 5 pairs for a bottle culture. After 7 or 8 days, when the culture is developing well, remove and discard the parents.

Each team should also make a new culture of the double mutant stock at the time the crosses are made so that virgin females will be available to cross with the F_1 males from the above matings.

When the F_1 flies emerge, in 10 to 12 days, examine at least 50 of them and record the phenotype. If more than 1 type is observed, continue to classify until 250 flies have been recorded. Each fly should be examined for both characteristics, so there are 4 possible combinations of characteristics: wild type (nonsepia and nonspineless), sepia nonspineless, nonsepia spineless, and double mutant (sepia and spineless).

Collect virgin females from the F_1 generation and mate them with double mutant (sepia spineless) males from the stock culture. Also mate F_1 males with virgin females from the double-mutant stock. After 7 or 8 days remove the parents of these test crosses from the culture vials.

When the test-cross progenies begin to emerge in 10 to 12 days, examine them and classify at least 200 offspring from each cross, but do not extend the count past the ninth day after the first flies emerge. Record the kinds

MATERIALS
Alternative A
Stock cultures of *Drosophila* as follows:
 a. Wild type (+)
 b. Sepia spineless (se ss) or brown vestigial (bw vg)
 c. Sepia nonspineless (se Ss) or brown

 nonvestigial (bw Vg)
 d. Nonsepia spineless (Se ss) or nonbrown vestigial (Bw vg)
Four fresh culture vials or bottles per team
Basic materials as in 30-1

of offspring and their numbers on a data sheet as directed by the teacher. How many classes of progeny do you obtain? (1) Do you get the same results from the test cross of virgin F_1 females as from the test cross of F_1 males? (2) Do your results agree with those obtained by the other teams who made the initial cross in a different way? (3) In the test-cross progeny derived from F_1 females, compare the frequencies of the classes carrying the parental combinations of the mutants with those carrying the mutants in recombinations. For example, from an original cross of sepia spineless by wild type, the possible recombinations in future generations are sepia nonspineless and nonsepia spineless. If the 2 mutant genes segregate independently, a test-cross ratio of $\frac{1}{4} : \frac{1}{4} : \frac{1}{4} : \frac{1}{4}$ is to be expected. Explain. (4) Test your results by the chi-square method (see Appendix) to see whether they agree or disagree with this expectation. Which is the case? (5) Which are more frequent, the parental combinations or the recombinations? (6) Is this also true in the reciprocal cross? (7) Are the 2 parental classes equal in numbers? (8) Are the 2 recombinant classes equal? (9) Pairs of genes carried on the same pair of chromosomes are said to be linked. Can you express the nature of this relationship by putting it in terms of the parental combinations as compared with recombinations? (10) Calculate the frequency of crossing over which gives rise to the recombinant classes. (Add the number of flies in the 2 recombination classes together and divide by the total number of flies classified.) What is it? (11) The locations of different mutant genes that give low frequencies of crossing over are thought to lie close together in the same chromosome pair, the loci of those that recombine more frequently lie farther apart. Compare the frequencies of crossing over calculated (a) when the 2 mutant genes were originally together in the same chromosome, and (b) when the 2 mutant genes were originally in separate, homologous chromosomes. Are the values practically the same? (12) Explain this. (13)

The F_1 is expected to produce 4 kinds of gametes (because it is doubly heterozygous). Let us designate the sepia gene by se and its wild-type allele by Se, and the spineless gene by ss and its wild allele by Ss. The gametes from an F_1 can be expressed as follows: $(pSs\ Se + qSs\ se + rss\ Se + tss\ se)$ in which $p + q + r + t = 1$. If, as in the previous exercise, the 2 pairs of alleles are independent, $p = q = r = t = \frac{1}{4}$. But in the present exercise the

values for p, q, r, and t depend upon (a) the sex of the double heterozygote, (b) the way the pairs of alternative traits were combined in the parents of the F_1, and (c) how far apart the genes are located on the chromosome.

All 4 teams made test crosses since the double mutant strain is homozygous recessive for both genes. Now that you have made the crosses and obtained data you will appreciate that the beauty and usefulness of this type of mating lies in the fact that the double mutant parent can produce only 1 type of gamete, that which carries both kinds of recessive alleles. This means that all differences in progeny are a reflection of differences in the gametes provided by the other (doubly heterozygous) parent.

From the results of reciprocal matings, compare crossing over in male and female *Drosophila*. (14) This difference between sexes is found in only a few organisms. It is an additional reason for *Drosophila* being so extensively used in genetic studies.

Alternative B:
Linkage and Crossing Over in Maize

PROCEDURE

In each team of two students let one person work as classifier of the seeds and the other as recorder. You may switch roles when halfway through. Mark the point of beginning at one end of a selected row by means of a pin or a spot of color that is later removable. Each seed is to be identified for all three alternative characteristics. That is, is it colored or colorless (Cc or cc, respectively)? Is it smooth and full or is it shrunken ($Shsh$ or $shsh$, respectively)? Is it starchy and dull in appearance or is it waxy and glistening ($Wxwx$ or $wxwx$, respectively)?

There are 8 possible combinations of 3 pairs of alternatives. The recorder must therefore prepare in advance a record sheet with 8

columns (or 8 rows) in which each of the possible kinds of seeds can be tallied. Start as follows: colored nonshrunken nonwaxy; colored nonshrunken waxy; colored shrunken nonwaxy; etc. After the 4 classes of colored seeds, write down the 4 classes that are noncolored.

If there is independent assortment, that is, if there is no linkage between the 3 pairs of genes, what ratio of the 8 classes would you expect to obtain in a test cross? (1)

After you are sure you can distinguish each trait, classify 200 seeds. Add up the total number in each class. If there is linkage between the 3 pairs of genes, then the particular combinations present in the chromosomes of the triple heterozygote (F_1 parent) should be obtained in the offspring more frequently than those that can only arise through crossing over. If you find 2 reciprocal classes (that is, classes that have exactly opposite traits, such as colored nonshrunken nonwaxy and noncolored shrunken waxy) which are much commoner than the other classes, this will identify the parental combinations of the genes. What are they? (2) Write down the parental combinations opposite a and b, group 1, in the table at the end of this exercise, to indicate the three traits that are linked. For example:

a. C Sh Wx   b. c sh wx, or
a. C sh wx   b. c Sh Wx, etc.

Group the 8 classes into 4 pairs of reciprocal classes, in the following way:

1. Parental combinations (both a and b)
2. Recombinations between C and Sh (both a and b)
3. Recombinations between Sh and Wx (both a and b)
4. Recombinations between both C and Sh and between Sh and Wx (both a and b)

Enter the number of seeds of each class in the column of the table headed "Number." Add together the number of seeds of the reciprocal classes in each of the 4 pairs, and enter these totals in the table under "Number per Group." Now add together all the recombinations between C and Sh (groups 2 and 4); and divide this sum by the total number of seeds classified to obtain the percentage of recombination between C and Sh. Then add together all the recombinations between Sh and Wx (groups 3 and 4); and divide this sum by the total number of seeds classified to obtain the percentage of recombination between Sh and Wx.

Between which genes is there greater recombination, between C and Sh or between

FREQUENCY OF CROSSING OVER

			Number	Number per Group	Percentage
Group 1:	Parental combinations				
		a.			
		b.			
Group 2:	Recombinations between C and Sh				
		a.			
		b.			
Group 3:	Recombinations between Sh and Wx				
		a.			
		b.			
Group 4:	Recombinations between both C-Sh and Sh-Wx				
		a.			
		b.			
		Total	200		

Sh and *Wx*? (3) Compare your percentages with those of other teams using the same three pairs of characters. (4)

To determine whether the order of the 3 pairs of genes on the chromosome is as we have been writing it, one applies the axiom that the whole should be equal to the sum of its parts. In other words, recombination between *C* and *Wx* should be equal to the sum of the recombinations between *C* and *Sh* and between *Sh* and *Wx*. Calculate the recombinations between *C* and *Wx* by simply ignoring *Sh*. This amounts to adding the number of seeds of groups 2 and 3 in the table. Calculate the percentage of this type of recombination. Is it larger than the percentage of recombination between *C* and *Sh*? (5) Is it larger than the percentage of recombination between *Sh* and *Wx*? (6) Is it about equal to the sum of the percentages of recombination between *C-Sh* and *Sh-Wx*? (7)

Fill in at the end of the exercise the map of the chromosome carrying these 3 genes. Put the locus of one of the genes involved in the largest recombination value (*C*?) at the zero end of the scale. Make the distance between the 3 loci proportional to the amount of crossing over you have observed to take place between them. An appropriate scale would be to let 2 mm equal 1 crossover unit, that is, 1% of crossing over. What is the distance in millimeters between the *C*, *Sh*, and *Wx* loci? (8)

The recombination actually found between the traits at the two ends of the map may not be quite as great as the sum of the recombinations between each of these and the trait in the middle. Can you suggest why this should be so? (9)

MAP OF C, Sh, AND Wx LOCI

0   140

Distance in millimeters

SEX-LINKED INHERITANCE

A departure from ordinary Mendelian behavior occurs whenever the genes for a pair of alternative traits are carried on the chromosomes that determine sex. In the fruit fly, *Drosophila*, there is one pair of sex chromosomes. In a female the two sex chromosomes of the pair are alike and are known as X chromosomes. A male has one X chromosome, just like those in the female in size and shape and general gene content, but in place of the second X chromosome there is a smaller, hook-shaped chromosome known as a Y chromosome. In meiosis in the male, the X and Y chromosomes pair together and then segregate from one another, just as X chromosomes pair and segregate in meiosis in the female.

■ The purpose of this exercise is to determine the type of inheritance that occurs when certain genes are carried on the sex chromosomes.

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MATERIALS

Drosophila stocks of wild type and white
  eye color
Four fresh culture vials or bottles
Basic materials as in 30-1
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PROCEDURE

Cross three white-eyed virgin females with three wild-type males in one culture. Make the reciprocal cross in the other vial—that is, three wild-type virgin females with three white-eyed males. Label each culture carefully with the type of cross and the date. After 7 or 8 days, examine to see that the cultures are developing and, if so, remove and discard the parents.

Examine the F₁ flies from each culture when they begin to emerge in 10 to 12 days. Classify each fly by eye color (red or white) and sex. Record the data in a form indicated by your teacher. Classify at least 70 flies from each cross.

Cross the F₁ females and males from each of the two reciprocal crosses. The females need not be virgin. Again, use three females and three males per culture; examine and remove the parent flies after 7 or 8 days; and classify the offspring, when they emerge, for eye color and sex. Classify at least 120 F₂ flies from each cross. Record the results in your chart.

From the original cross of wild-type females with white-eyed males, which would you conclude to be the dominant trait, red eye color or white eye color? (1) If this is so, how do you account for the result obtained in the reciprocal cross of white-eyed females by red-eyed males? (2) Does the Y chromosome carry any allele of these eye-color genes? (3)

Record these results in a chart form which your teacher will suggest. Use X and Y with a superscript to represent the gene. If red eye color is recessive to white eye color, use an r for red and an R for white. If white eye color is recessive to red, use a w for white eye color and a W for red eye color. Thus, the original white-eyed females would be $X^R X^R$ if you find that white eye color is dominant, but you would write $X^w X^w$ for them if you find that white eye color is recessive. For the Y chromosome, write Y^R or Y^w if you conclude that the Y chromosome carries eye-color genes, but if it does not seem to do so, use the symbol Y- to indicate that it is "empty" so far as this locus is concerned. Determine what kind of gametes each parent type can produce, and what combinations of offspring are to be expected in each generation, both for the F₁ and the F₂ generations. For each type of *individual*, besides the genotype write also its phenotype, that is, *red eye* or *white eye*.

What condition must exist to permit white-eyed females to occur? (4) If an F₁ female from either of the original crosses is bred, will it matter what kind of male she is mated with insofar as her sons are concerned? (5) Insofar as her daughters are concerned? (6)

GENETIC DIFFERENCES IN PEAS

Differences in organisms are brought about by the ways in which environment modifies the development of different inherited possibilities. They may appear as clear distinctions in appearance or size, as microscopic variations in structure, or even as invisible chemical differences showing themselves in any number of ways in the organism's metabolism. In each case, the genetic information inherited by the organism forms the basis for the eventual manifestation.

■ The purpose of this exercise is to study round and wrinkled peas to show that differences that appear to be superficial or trivial may, in fact, probably be associated with biochemical and enzymatic distinctions of great importance.

MATERIALS

Peas of round and wrinkled genetic strains
Two small bottles
Microscope slide
Razor blade or scalpel
Compound microscope
Paper towel
Wax pencil
One dish of glucose agar
Mortar and pestle
300 ml distilled water
Two 250-ml beakers
Two medicine-dropper pipettes
Iodine solution for starch tests
Balance
Cheesecloth or centrifuge
Blotting paper

PROCEDURE

Look at your sample of 50 dried peas evenly divided between round ones and wrinkled ones. First we should determine whether this obvious difference between the two types is the result of different genes, or whether it has perhaps been produced by uneven drying methods. To do this, "in reverse," we may measure the amount of water that can be soaked up by each kind of peas.

Mark each of two bottles with your name and the kind of peas contained. The bottles should be large enough that the dried peas, of one kind, will not fill more than half its capacity. Rinse each bottle and shake out the larger drops. Weigh, and record its weight in a table that your teacher gives you. Then add one kind of peas and re-weigh peas and bottle together. Record the weight in the appropriate place in the table. Do the same for the second bottle with the other kind of peas. Calculate the weight of each group of peas and enter these weights in the table. Now fill each bottle with water and set it aside to stand overnight.

After soaking the peas overnight, pour off the excess water from each bottle, empty the two kinds of peas into separate piles on a piece of paper towel and shake out the larger drops of water from the bottle. Return the peas to the proper bottle, and again weigh peas and bottle. Record these weights and complete the calculations in the table.

What can you conclude about the amount of drying and the appearance of the two kinds of dried peas? (1)

Starch Grain Formation

Mark one end of a microscope slide with w for wrinkled and the other end with R for round. Place a drop of water at each end. Cut through a soaked wrinkled pea with a razor blade and gently scrape the cut surface into the appropriate drop of water, mixing the scrapings well into the drop. Clean the razor blade in running water and cut through a soaked round pea, working its scrapings, as before, into the proper drop of water.

Using the low power of the microscope, look at the two drops with the scrapings. Make simple outline drawings of the shapes of the starch grains in each drop. Describe the differences

between the starch grains from round and from wrinkled peas. (2) Are some compound? simple? whole? divided? oval? round? Look at a few slides prepared by other students in the class. Are the preparations consistently the same as yours? (3)

Carbohydrate Synthesis

An important feature in the metabolism of any green plant is its synthesis of food. The pea makes large starch molecules from simpler and smaller sugar molecules with the help of its starch-forming enzyme.

To see whether there is any difference in the starch-making capacities of the round and wrinkled peas, we may extract the starch-forming enzyme from each type and add equal amounts separately to a supply of a simple sugar (glucose), which can be converted into starch. After a suitable lapse of time, an iodine test can be made for the presence of starch, and the results then compared.

Work in teams of four students. Each team is to weigh out 10 g of dried round peas and 10 g of dried wrinkled peas. Grind the round peas in 10 ml of water with mortar and pestle until no big particles remain. (This takes a great deal of effort.) Filter the mixture through a layer of cheesecloth into a small beaker; or better, centrifuge the mixture to obtain a clear solution free of sediment. This clear solution is our "enzyme extract" to be tested for its capacity to make starch from sugar. Mark the beaker ROUND PEAS, and add the designation of your team. Repeat the procedure with the 10 g of wrinkled peas.

Remove the cover from a Petri dish containing agar and glucose. Divide the plate into right and left halves by drawing a line, with a wax pencil, on the *outside* of the bottom dish. Why not mark on the cover? (4) On the left side of the agar plate place four separate drops of the enzyme extract from the round seeds; on the right side place four separate drops of en-

zyme extract from the wrinkled seeds. Mark these sides R and W on the bottom of the dish. The four enzyme-extract drops on each side should be well separated from one another. Allow the enzyme to act for 30 minutes on the glucose in the agar. Then add a drop of iodine test solution to each of 2 drops of the enzyme extract from the round peas, and to each of two drops of the enzyme extract from the wrinkled peas. Look for the appearance of the blue color of starch beneath the surface of the agar. Compare, and record the results in the table which your teacher will supply. (Unless the extract was centrifuged, it probably contains a few starch grains, and these should be removed. So gently blot off the drop of enzyme extract and iodine with a small piece of absorbent paper and then, after the surface starch has been removed, look for the evidence of starch-making *underneath* the surface of the agar.)

Wait another 30 minutes, then test the remaining two drops from each extract with iodine solution, as before. Record results in the table.

What differences between the extracts from round and wrinkled peas are observed? (5)

Assuming that the round and wrinkled peas developed and were dried under very similar conditions, what might account for the differences you find here? (6)

You have seen differences between peas at three different levels of observation: (a) the rather crude, surface distinction of round and wrinkled skins; (b) the microscopic contrast between the starch grains in the two kinds of peas; and (c) the chemical reactions connected with differences in the enzymes possessed by round and wrinkled peas. These two types of peas can be shown, by breeding experiments, to depend on a difference in a single gene.

Which of these three levels of observation that you have used comes closest to distinguishing the primary action of the gene? (7) Why do you think so? (8)

NUTRITIONAL MUTANTS

IN *NEUROSPORA*

Early in this century geneticists already believed that genes somehow controlled the production of enzymes. If a particular gene were altered, then the enzyme it produced would be changed either in amount or kind, and this would eventually lead to a phenotypic change that we could detect. This was all very logical; but no one had detected an enzyme that was gene-produced, much less a change in an enzyme that had resulted from a change in a gene.

There are so many developmental steps between a gene with its supposed enzyme and the observed phenotype in complex organisms, such as *Drosophila* and corn, that George W. Beadle and E. L. Tatum turned to the ascomycete *Neurospora* (a mold) in an attempt to find the primary effect of a gene. (Beadle and Tatum received a Nobel prize in 1958 for their work on this problem.)

Beadle and Tatum found that they could grow the wild (+) form of *Neurospora* on a very simple agar medium containing only certain inorganic salts, sucrose, and the vitamin biotin. This is called a minimal medium, since no medium with fewer components will support growth.

They reasoned that the organism itself must build up all the more complicated molecules, such as proteins and nucleic acids, from inorganic salts, sucrose, and biotin. Such biochemical syntheses require many enzymes, and the plant must have all of these enzymes. If geneticists were correct in their belief, at least some of these might be gene-controlled.

If a mutation occurred of such a nature that a certain enzyme was no longer produced, the reaction for which this enzyme was necessary would not take place and the resulting compound would not be produced. If this were a necessary compound, the plant would die unless it were supplied in the medium, since the plant itself could not make it.

■ The purpose of this exercise is to trace the experimental steps that led Beadle and Tatum to their Nobel prize-winning discovery of the primary effect of a gene.

MATERIALS: None

Life Cycle of an Ascomycete

Ascomycetes reproduce either sexually or asexually (vegetatively). Sexual reproduction starts with fusion of the hyphae of the two sexes (called A and a). The nuclei in the hyphae are monoploid, so fusion of an A nucleus with an a nucleus results in a zygote or diploid nucleus. This diploid nucleus immediately undergoes meiosis, and the resulting monoploid spores give rise to the vegetative hyphae.

Production and Recovery of Mutations

It is possible to irradiate with X rays or ultraviolet rays some of the vegetative stages and thus obtain many mutations. The irradiated vegetative form is then crossed with the wild type of the opposite sex. The resulting spores can be separated and individually cultured.

These spores, which might have mutations, are first cultured on a complete medium, each spore in a separate culture tube. The complete medium contains all sorts of amino acids, vitamins, purines and pyrimidines, as well as salts and sucrose. Some of the mold growth from each tube of complete medium is then transferred to a minimal medium. In some tubes of minimal medium there will be further growth of the mold, but in others, no growth. Why the difference? (1) What can you infer about the nutritional requirements of those forms having no growth on the minimal medium? (2) Why were the spores first cultured on a complete medium? (3)

Identification of a Nutritional Requirement

If there is no growth on the minimal medium after normal growth on the complete medium, some of the growth from the complete medium is transferred to each of two special media. One of these contains just the vitamins, in addition to the constituents of the minimal medium; and the other medium contains the 21 different amino acids known to be in the proteins of *Neurospora*, in addition to the minimal constituents.

Let us follow an actual experiment. A number of spores gave rise to mutant cultures that would not grow on minimal medium. Some of these would grow on minimal medium plus vitamins but not on minimal medium plus amino acids. What do you conclude about these mutants? (4) Others would grow if the amino acids were added but not if vitamins only were added to the minimal medium. What is your conclusion about these mutants? (5)

We will follow the remaining steps of the procedure on one mutant of the second group (those needing some amino acid). A series of media, each composed of the minimal medium and one of the amino acids was inoculated from the culture growing on complete medium. Growth was found in only 1 of the 21 cultures, and that culture had *arginine* added to the minimal medium. What was the effect of the mutation we have been testing? (6)

In a very large experiment a number of mutant strains were obtained that would not grow unless given some arginine. Arginine is one of the naturally occurring amino acids found in almost all proteins. Ornithine and citrulline are closely related chemically to arginine but are not found in protein. Because of this chemical similarity, the mutants that needed arginine were then tested on minimal medium plus ornithine, and minimal plus citrulline. Some mutants, for example, were found to use citrulline for growth as well as arginine. The following table, Growth of Mutant Strains on Various Media is adapted from a research paper published by Adrian Srb and J. Horowitz in

GROWTH OF MUTANT STRAINS ON VARIOUS MEDIA

Mutant Strain	Minimal Medium No Supplement	Dry Weight Values in Milligrams		
		Orni-thine	Citrul-line	Argi-nine
1	0.0	0.0	0.0	20.4
2	1.0*	0.8*	34.1	37.6
3	2.3*	2.5*	42.7	35.0
4	0.9*	29.2	37.6	37.2
5	1.1*	25.5	30.0	33.2
6	0.0	10.5	18.7	20.9

* Values of 2 5 or below are considered as "no growth." Such slight growth is not significant for purposes of this experiment

the *Journal of Biological Chemistry* in 1944. The table gives a partial list of mutants and their growth on various media.

You will notice that the data in the table are arranged in an orderly manner. Such an arrangement is possible only if there is some intrinsic biological organization of the chemical steps involved. By discovering the intrinsic pattern, the investigators developed a hypothesis concerning the order of activity of a number of genes in the wild type.

Can Strain 1 grow on minimal medium? (7) Can Strain 1 grow if arginine is added to the minimal medium? (8) Can ornithine or citrulline take the place of arginine as a supplement to the minimal medium for Strain 1? (9)

Can Strain 2 grow on minimal medium? (10) If arginine is added will Strain 2 grow? (11) Can citrulline replace arginine as a supplement to the minimal medium for Strain 2? (12) What might you imagine Strain 2 does with citrulline? (13) Compare the growths of Strains 1 and 2 with arginine and citrulline. Can you devise a scheme for the step-by-step order of action of the genes that mutated in these two strains? (14)

Now devise a scheme incorporating all six mutants. (15)

A POPULATION GENETICS STUDY

In this exercise we will use the occurrence of an obvious and easily determined trait in order to calculate gene frequencies in a human population.

The inherited ability to roll the tongue is an excellent characteristic for a population genetics study—that is, a study to find out, first, how common the trait and its opposite (inability to roll the tongue) are in a representative population; and second, what the frequencies of the alternative alleles are in the population. It is, of course, quite impossible to determine the presence or absence of the ability in all members of a sizable population, so we must resort to sampling. Our sample ought to be made up as randomly as possible in order to be truly representative of the population from which it is drawn. It should also be fairly large because, if a sample is small, chance may make it unrepresentative. In a properly random sample of a population, members of the same family ought not to be included any more frequently than if they were chosen by chance from the population. Since the latter would happen with only a low frequency, it is better to make the simple rule that in no case will a parent and offspring both be included in the sample. This is not quite random because a few such cases should be included, but it avoids a greater deviation from randomness in the opposite direction, which would most likely be the case if such a rule were not imposed. If there is any reason to believe that the trait being sampled affects the choice of marriage partners, then both man and wife should not be included in the sample.

■ The purpose of this exercise is to discover how common a given trait is in a representative human population and what are the frequencies of the alternative alleles in the population, according to the assumptions of the Hardy-Weinberg Principle.

PROCEDURE

A. Probably no two students belonging to the same family will be members of the same class in school. Therefore, let us begin by determining the frequency of tongue rolling among the members of our class; this should give us a small, but fairly random, sample of our local population. From Figure 33-1-1, familiarize yourself with the tongue-rolling trait. Determine whether you can do this. Most persons find that either it is very easily done, without any need to practice, or else it cannot be done at all after any amount of trying. Record the results in your notes. When everyone has done this, pool the results and record them on a form similar to the following:

Number of Tongue Rollers	Number of Nonrollers	Total	Percentage of Rollers

B. To gather further data, determine the number of tongue rollers and nonrollers in your own immediate family (parents, brothers, and sisters). Record the results. When these are reported back to the class, and all the data from all the families are pooled and recorded, as above, is the percentage different from that in the class itself? (1) Now, from the several families, determine whether tongue rolling is dominant or recessive. *Clue:* If it is dominant, a marriage of two tongue rollers may produce children who cannot roll the tongue. If tongue rolling is recessive, a marriage of two tongue rollers produces only tongue-roller children. Which seems to be the case? (2) Are the crucial families large enough to make the answer certain? (3)
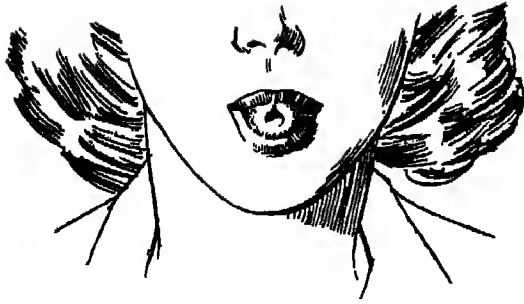
Figure 33-1-1 Tongue rolling.

C. Now, in a further extension of data gathering, every student should obtain data on tongue rolling from five neighbors, none of whom belong to the same family. (Husbands and wives, however, may both be included.) If you live near another classmate, arrange among yourselves to sample different families. It is very important *not* to get data from more than one person per family (except husbands and wives) in the entire sample collected by the class. Report the data and again compare the total number of tongue rollers with that in the class, and with that in the families. Is it about the same, or quite different? (4) Add together the class data and the data from neighbors, but do not add in the family data. Calculate the percentage of tongue rollers in this new total.

D. According to the Hardy-Weinberg Principle, if the population sampled is in a genetic equilibrium with respect to the trait being studied, then the ratio of genotypes for a simple two-allele difference is $p^2 : 2pq : q^2$, where p and q are the respective frequencies of the dominant and recessive alleles. If you have determined whether tongue rolling is dominant or recessive, note the frequency (percentage) in the random sample of the population of the *recessive* trait. This is equal to q^2. Take the square root of this percentage to find q. For example, if the frequency of the recessive trait is 36% (0.36), its square root will be 0.60, or 60%. If the frequency of the trait is 16% (0.16), its square root is 0.40 or 40%. Is the frequency of the recessive allele larger or smaller than the frequency of the recessive trait? (5)

Having determined the value of q, the recessive allele, now find the value of p. If there are only two alleles, $p + q = 100\%$, or 1.00. Therefore, $p = 1.00 - q$. (For example, if $q = 0.60$, $p = 0.40$. If $q = 0.30$, $p = 0.70$.)

Now substitute the values of p and q in the binomial expression, $p^2 + 2pq + q^2$. You already know the frequency of the recessive trait, which is q^2. Find the frequency in your population of the homozygous dominant type (p^2) and the frequency of the heterozygous type ($2pq$). What is the ratio of the homozygous dominant type to the heterozygous type, that is, $p^2 : 2pq$? (6)

In a single family in which each of the two parents are heterozygous, the offspring are expected to be, according to simple Mendelian laws, three of dominant type to one of recessive type. In the population sample you have analyzed, is the ratio of the dominant persons ($p^2 + 2pq$) to the recessive persons (q^2) a $3/4 : 1/4$ ratio, or not? (7) Explain.

E. We may also calculate the probability of marriages between persons of the three genotypes. Is choice of mates likely to be affected by the trait in the case of tongue rolling? (8) If choice of mates is not affected by the trait, the probability of any particular mating is the product of the frequencies of males with the specified genotype and females with the specified genotype. For example, suppose some population is made up of 49% homozygous brown-eyed persons, 42% heterozygous for eye color, and 9% homozygous blue-eyed persons. Are these distributed according to a Hardy-Weinberg expectation? (9) If eye color does not affect choice of mates, the probability of a marriage between a blue-eyed person and a brown-eyed person would be *double* $(0.49 + 0.42) \times 0.09 = 2 \times 0.91 \times 0.09 = 0.1638$, or about 16% of all marriages. Why double? Because blue-eyed by brown-eyed matings could be either blue-eyed female by brown-eyed male, or blue-eyed male by brown-eyed female. Now calculate for tongue rolling. In your population, how often would you expect a tongue roller to marry a nonroller? (10) Other frequencies of matings may also be predicted.

HUMAN INHERITANCE

■ The purpose of this exercise is to determine how a sex-linked factor is transmitted in a single family and then to determine the frequency of the alternate alleles in the general human population.

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MATERIALS: None
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Part A:

Sex Linkage

When a gene is carried on one of the chromosomes that determine sex it must, of course, go wherever the sex-determining chromosome goes. We speak of the gene as being "linked" with the sex-determining chromosome. If a gene is carried on the X chromosome in a male, it has no allele in the much smaller Y chromosome (at least in all of the cases we shall consider). Consequently, in a male, a recessive trait controlled by a gene on the X chromosome will show up although just one recessive

allele is present. In a female, both alleles must be recessive for a recessive trait to show.

Since experimentation is not possible in human genetics and pure (breeding) races or strains are only "pure nonsense," understanding the mode of inheritance of human traits depends almost exclusively upon finding and analyzing the appropriate pedigrees.

Red-green color blindness is one human trait the genetics of which is well understood. From the following pedigrees you should be able to determine which allele (for normal color vision or for color blindness) is dominant and whether or not the gene is sex-linked.

First a sample pedigree (Figure 33-2-1) is given to explain the symbols.

In Figure 33-2-2 on color blindness, if a circle or square is filled in, ● or ■, that individual has the trait in question.

Both of these two pedigrees relate to the *same* trait, that is, red-green color blindness. Is the allele for color blindness dominant or recessive? (1) Is it sex-linked or not? (2) Point out the evidence for your answers to the last two questions. (3)
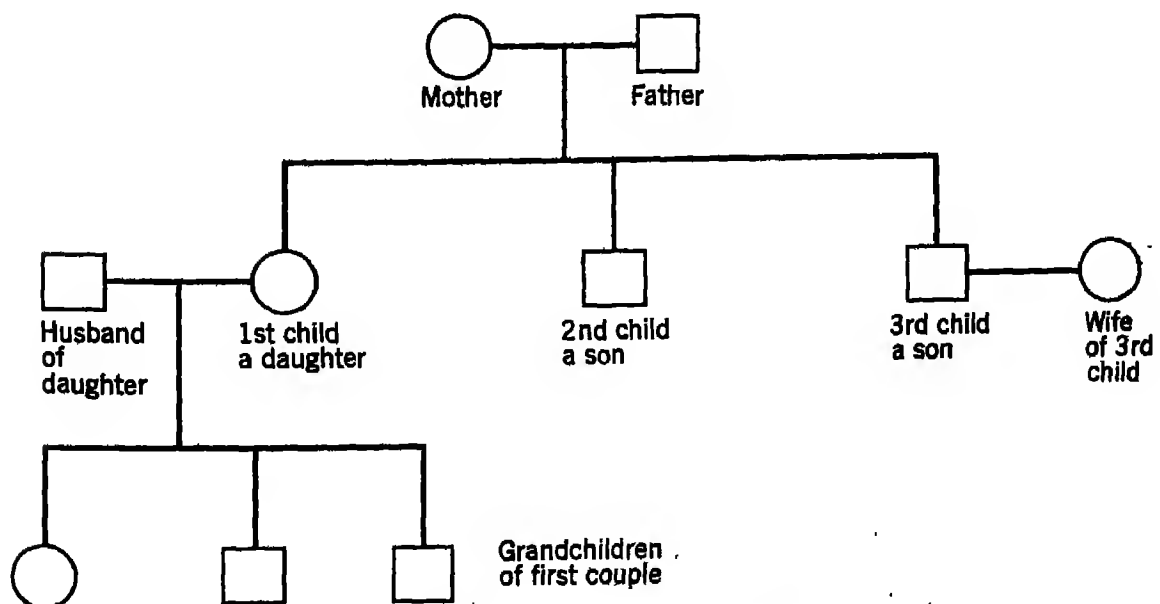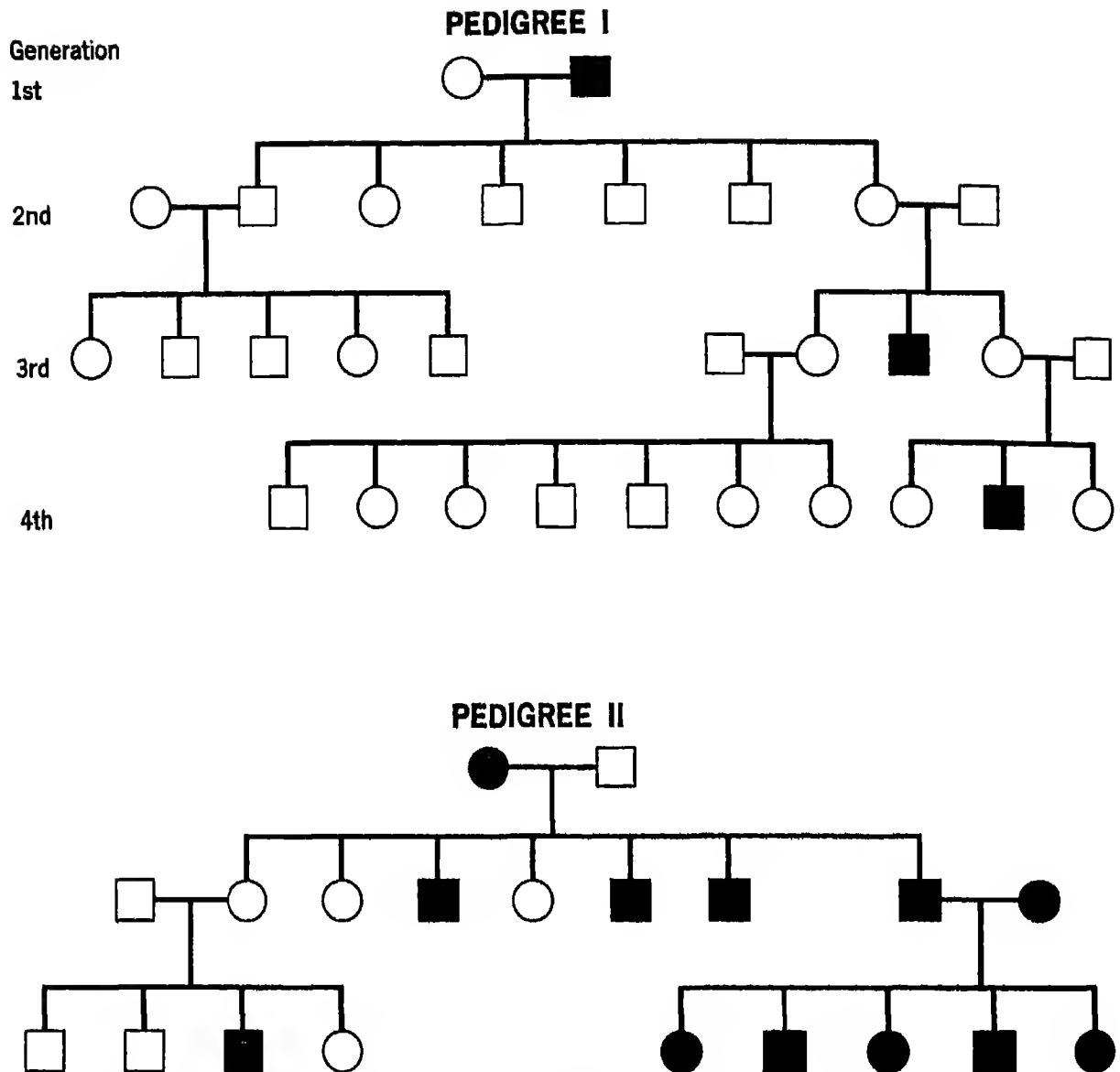

Figure 33-2-1 A sample pedigree.

PEDIGREE I

PEDIGREE II

Figure 33-2-2 Two pedigrees of color blindness.

Now let us predict the expected results of a mating between a color-blind man and a woman homozygous for normal color vision.

Among their male offspring, what fraction have normal color vision? (4) What fraction are color-blind? (5) Among their female offspring, what fraction have normal color vision? (6) What fraction are carriers? (7) What fraction are color-blind? (8)

Repeat the calculations, this time for a mating between a carrier woman and a man with normal color vision.

Among their male offspring, what fraction have normal color vision? (9) What fraction are color-blind? (10) Among their female offspring, what fraction have normal color vision? (11) What fraction are carriers? (12) What fraction are color-blind? (13)

Another sex-linked character is hemophilia. The gene for this condition, in which blood clots extremely slowly, is carried on the X chromosome. As in color blindness, it takes but one recessive allele for a man to be hemophilic, and it takes two to produce the condition in a woman.

Figure 39-1 in the textbook shows, in part, the distribution of hemophilia among certain royal families of Europe (data incomplete). Remember that circles stand for females and squares for males. Study the chart and then list those mothers who *must* have been carriers. (14)

The genes for red-green color blindness and hemophilia are located about 10 crossover units apart on the X chromosome; that is, crossing over between them occurs in about 10% of

the gametes. A few families have been discovered in which both the recessive allele for color blindness and the recessive allele for hemophilia were present in the same woman. Assume that her husband is free of both these inherited defects. Assume also that she has one recessive in one X chromosome and the other recessive in the other X chromosome; in other words she is *Hc/hC*. What does this information tell you about the possible genetic make-up of her father? (15) Which possibility is more probable in her father? (16) What types of sons can be produced in this family? (17) What is the probability for each kind of son? (18) What kinds of daughters can be produced in this family? (19)

Part B:
Human Population Genetics

Let us now try to find out, not how color blindness is transmitted in a single family, but rather what frequency it has, and what frequency the recessive allele for color blindness has, in the general population. We will start with a *school sample* of the population, compare the results with a larger *community sample*, and then from our findings calculate what the frequency of occurrence of color blindness should be in the general population. We may also calculate the frequency of the dominant *C* and recessive *c* alleles of each particular genotype by applying the Hardy-Weinberg Principle.

PROCEDURE

The color-blindness chart in the Appendix can be used to make a preliminary determination of normal color vision or of red-green color blindness. People with normal color vision will read the word "onion," and those who are red-green color-blind will see the word "color." Determine what color vision you have and report the findings to your teacher. Summarize the findings of the whole class in a table that your teacher will show you how to set up.

Now test as many members of your family as possible. Make a genealogical chart for them, females represented by circles, males by

squares, and those who are color-blind blacked in. Locate yourself with an arrow.

Also test ten people (excluding anyone tested by another member of your class) who are genetically unrelated. This means that man and wife can both be counted but not parent and offspring. (You can use your own parents as two of the ten.) Enter the data for the general population on a table that your teacher will give you.

What percentage of all the males in the class are color-blind? (20) In the total data? (21)

What percentage of all the females in the class are color-blind? (22) In the total data? (23) How well do these figures agree with the general estimate for the United States? (24) (Approximately 8% of the males and 0.6% of the females are color-blind in the U.S.)

Because the Y chromosome carries no allele for color blindness or normal color vision, examining a male's phenotype with respect to color vision is equivalent to examining the genotype of his X chromosome. By finding the frequency of color-blind males in a population we are, therefore, obtaining the frequency of the chromosomes with the allele for color blindness, or in other words the frequency of the allele *c* for color blindness. In males, therefore, p is the fraction of males with *C* on their X chromosomes and q is the fraction with the *c* allele and color blindness. And (pC + qc) = 1, that is, all the males there are.

If the ability to distinguish red and green is not involved in selecting one's husband or wife, we should expect marriages to be at random with respect to the alleles C and c. Then there will be the same frequencies of C and c in females as in males. But the phenotypic frequencies are different for the two sexes because of the different genotypes for sex-linked traits.

In terms of p and q, what is the expected frequency of CC women? (25) Of heterozygous women (also called carriers)? (26) Of color-blind cc women? (27)

From the data obtained by your class, determine the frequency of women who are carriers. What is it? (28) Hint: First determine the value of q from the observed frequency of color-blind males. Then find p = 1 − q, etc. Remember that a frequency such as 0.25 means the same as 25%.

Evolution

It would be nice, if for the purposes of this group of exercises we could take an animal species and watch it evolve by means of mutation and adaptations into another species. Unfortunately, however, the time required for such a process would extend for centuries at the very least.

However, it is possible in the following exercises to present you with some of the data that demonstrate the role of certain factors in the process of evolution. None of us can deny that changes take place in organisms through time, and it is even possible to observe seemingly miraculous changes in the laboratory in a short period of time, for example when a caterpillar changes into a butterfly or when a swimming, aquatic, legless, gilled, plant-eating tadpole changes into a jumping, land-living, lung-breathing, tailless, insect-eating frog. The purposes of the following exercises, however, are not so much to illustrate what changes actually have taken place, but rather to show the means whereby such changes have been produced. Working with data that demonstrate evolutionary principles will give you some understanding of the complexity of evolutionary processes and of how the factors of mutation, natural selection, and migration can be studied scientifically on an experimental basis.

SELECTION AND ALLELE

FREQUENCIES

In order to see what effect natural selection may produce on the allele frequencies (that is, the occurrence of particular alleles) in a population, it is first necessary to see what happens to allele frequencies from one generation to the next in the absence of natural selection or other disturbing factors. A disturbing factor might be, for example: the occurrence of mutations; the immigration or emigration of many individuals to or from a population; the effects of change in a population that is so small that such effects do not cancel one another out. A problem based on a real study of the frequencies of two types of moths belonging to a certain species and occurring together in the same population will help to make these relations clear.

■ The purpose of this exercise is to examine the frequencies of two alleles maintained in a population: first, when completely isolated from disturbing influences; and second, when under the pressure of natural selection favoring one type more than the other.

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MATERIALS: None
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Part A:
Gene Frequencies in Populations

To make the situation just as simple as possible, let us first consider an imaginary population of moths in which there is a constant population size and a constant environment. In this species of moth there are two hereditary phenotypes, one black in color, the other white speckled with black. Crosses have shown us that black W is dominant over speck-

led white w, and that the genes determining this difference are a simple pair of alleles.

Assume that the population consists of 1000 moths, with equal numbers of males and females, and that 500 are homozygous black WW and 500 are homozygous speckled white ww. Diagram the results of random mating in this population. Remember that black can mate with black as well as with speckled white; and that speckled white can mate with speckled white as well as with black. Will a black male have an equal chance of mating with a black female and with a speckled white female? (1) Will a speckled white female have an equal chance of mating with a black male and with a speckled white male? (2) Your diagram should show four possible kinds of matings. Now express these four kinds of matings in terms of genotypes; for example, $WW \, ♀ \times ww \, ♂$. (3) Alongside each type of mating, place the genotypes of all the kinds of offspring that that cross can produce. Does any cross produce more than one kind of offspring? (4) What will be the proportions of black and speckled white moths in the next generation, if we assume that it is also composed of just 1000 moths and that all four types of mating contribute equally to it? (5) Is the proportion of black and speckled white moths the same as in the population with which we started? (6)

Now consider the frequencies of the two alleles, W and w, in this population. What was the proportion (percentage) of each to start with? (7) What are the proportions in the second generation? (8) Are the proportions the same as in the first generation, or different? (9)

You can now carry the calculation to still another generation by mating every genotype with every other genotype *in proportion to their frequencies*, as shown in the following table—Frequencies of Black and Speckled White Alleles.

Parents (¼WW + ½Ww + ¼ww)		Offspring					
♀	♂	WW	Ww	ww	WW	Ww	ww
¼ WW	× ¼ WW →	1/16 (1)			1/16		
¼ WW	× ½ Ww →	1/8 (½ + ½)			1/16	1/16	
¼ WW	× ¼ ww →		1/16 (1)			1/16	
½ Ww	× ¼ WW →	1/8 (½ + ½)			1/16	1/16	
½ Ww	× ½ Ww →	¼ (¼ + ½ + ¼)			1/16	2/16	1/16
½ Ww	× ¼ ww →		1/8 (½ + ½)			1/16	1/16
¼ ww	× ¼ WW →		1/16 (1)			1/16	
¼ ww	× ½ Ww →		1/8 (½ + ½)			1/16	1/16
¼ ww	× ¼ ww →			1/16 (1)			1/16

Frequencies of the particular parents in the population

A fraction in front of a parenthesis indicates the frequency of a particular mating; that is, the product of the frequencies of parents. Fractions inside parentheses give the distribution of genotypes within offspring from that particular kind of mating.

¼ WW + ½ Ww + ¼ ww

Total offspring population

Notice that the frequencies of the three genotypes in the offspring population are the same as they were in the parent population. Compare the allele frequencies in the parent population with those in the offspring population. (10)

You can obtain the genotypic frequency of the offspring population by the much easier gene-pool method, if (a) you compute the frequencies in the population of all the kinds of gametes relating to the character under study, and (b) then assume random combination of these gametes. For example, if the frequency of W in the population is 0.5 and of w is 0.5, then half the sperms will be carriers of W and half will be carriers of w; and the same holds true for the eggs.

Hence the result will be:

$$\begin{array}{rl} \text{Sperms:} & 0.5\ W + 0.5\ w \\ \text{Eggs:} & \times\ 0.5\ W + 0.5\ w \\ \hline \end{array}$$

Product = 0.25 WW + 0.50 Ww + 0.25 ww offspring generation

Notice that the results obtained by the two methods, (a) compiling the offspring of random matings, and (b) gene-pool method, are identical.

The preceding example is very special since $p = q = 0.5$. It is important to see whether or not the conclusion we have drawn will also follow, regardless of what the original frequencies of W and w might be. Go back to the beginning and repeat the calculations using both methods; but this time assume that there are 400 homozygous black and 600 homozygous speckled white moths in the population. What do you find? (11) Try to state in a few sentences your general conclusion from the full study up to this point. (12)

Part B:
Natural Selection

Examine the pictorial story (Figure 34-1-1). It is purely a hypothetical model and not an actual situation that occurred. State what change took place in the environment of the original moth population. (13) What change was produced in the moth population as a result of this environmental change? (14) Cite the evidence that indicates that the change in the moth population is not simply a phenotypic effect of the environment, but is really a hereditary population change. (15) Do you think that a change in reproductive capacity of the two kinds of moths or a change in selection pressure resulted in the separation of the original homogeneous moth population into two distinct subpopulations? (16) What has happened to the frequency of the allele for

NATIVE WOODS CONTAINING PINE AND BIRCH TREES

Dark and white moths
on pine bark

50%

50%

Dark and white moths
on birch bark

Suppose 85 years later...

PLANTED WOODS OF PINE AND BIRCH ALONGSIDE FOUR-LANE DIVIDED HIGHWAY

96%

4%

85%

15%

PINE WOODS

BIRCH WOODS

Wings of white
moths

Wings of dark
moths

Dark moth lays eggs
on pine and birch

Larvae

Eggs

Dark moths produced

White moth lays eggs
on pine and birch

Larvae

Eggs

White moths produced

Figure 34-1-1   Pictorial story of selection pressure.

speckled white color in the moth population now living in the pine woods? (17) What has happened to the frequency of the allele for speckled white color in the moth population living in the birch woods? (18) If environments change over a period of time, what must happen to populations if they are to survive? (19)

If natural selection is responsible for the changes in frequency of black and of speckled white moths in the two types of woods, what comparison can you make between the color of the favored type of moth and the color of the bark of the trees in each woods? (20) Assuming there is benefit in protective coloration on the part of moths, what type of predators would you suspect to prey on moths? (21) What special abilities would these predators have to possess if they are really the agents of selection here? (22) Can you devise an experiment that would test this hypothesis? (23)

SICKLE CELLS AND SELECTION

What difference does it make if a man's red blood cells are oddly shaped? What causes the red blood cells of certain people to take on this peculiar shape?

These questions must surely have occurred to James B. Herrick in Chicago in 1910, when he examined the blood of a Negro boy with a mysterious disease. The red blood cells of the boy were crescent- or sickle-shaped. Soon after Herrick had written an account of his discovery other doctors uncovered other cases of the same illness.

Today we know that the sickling is due to a hemoglobin molecule slightly different from normal and that it is genetically determined. The disease associated with sickling is a severe anemia often leading to death in childhood.

■ The purpose of this exercise is to determine the population aspects of sickle-cell anemia and discover how it is inherited.

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MATERIALS: None
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Inheritance of Sickle-Cell Anemia

Diagnosis of the disease is made by putting blood into an atmosphere devoid of oxygen. It is under such conditions that extreme deformities in red blood cells from sickle-cell anemia patients occur. If the blood of the parents is examined, less extreme deformities are found in the red blood cells of both parents of a child with the anemia. These parents have no anemia but some sickling of the red blood cells may occur at high elevations or during prolonged physical exertion. This mild sickling without anemia is called sickle-cell trait.

Examine Figure 34-2-1. Is this pedigree consistent with the hypothesis that sickling of the red cells is a one-gene hereditary trait

in which there is no dominance? (1)

How many phenotypes are there in the family? (2) If only one gene with two alleles (S representing the allele for normal hemoglobin and S' representing the allele for sickle hemoglobin) is involved, how many genotypes might you expect? (3)

In the pedigree, what is the genotype of Individual 1? (4)

Notice that Individual 2 has very severe sickling and that many people with severe sickling die in infancy or childhood. Would you expect Individual 2 to have genotype $S'S'$ or SS'? (5) Individual 3? (6) Individual 4? (7) If Individual 5 marries a person with a genotype like her own, what percentage of their children would you expect to have sickle-cell anemia? (8) Sickle-cell trait? (9) Normal red blood cells? (10)

Population Genetics of Sickle-Cell Anemia

In certain African tribes of Negroes the sickle-cell trait may be found in as many as 40% of the persons in the tribe. The gene is not ordinarily found in white populations. If $4/10$ of the adult population are heterozygous (SS'), the chance of a heterozygote mating with a heterozygote is $4/10 \times 4/10$. Thus, $16/100 = 4/25$, approximately $1/6$ of the matings would involve two heterozygotes. There are almost no adult homozygotes. What fraction of the offspring in the population is then expected to be homozygous for the sickle gene? (11) Would you expect individuals with sickle-cell trait (SS') to leave, on the average, more or fewer descendants than those with normal red blood cells? (12) Would this result increase, reduce, or have no effect upon the total number of genes for sickling in the population? (13)

There is no evidence that the frequency of the gene for sickling is being reduced in the African populations. The question then arises: How can the gene for sickling be maintained
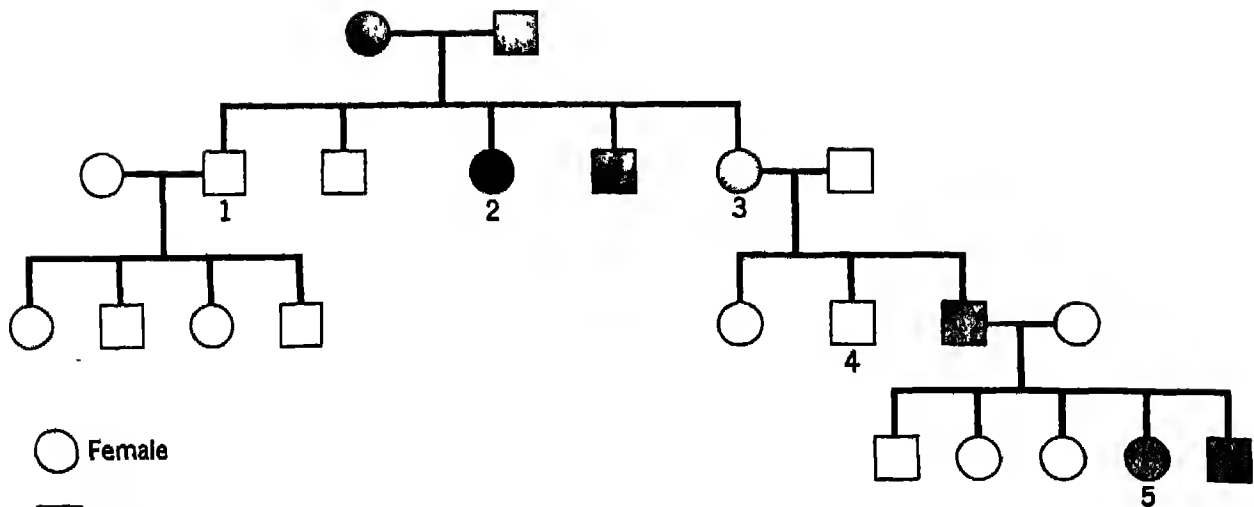
◯ Female

▢ Male

◯ Person with normal red blood cells

◯ Person with sickle-cell trait
(tendency to sickle when the individual
is deprived of a normal supply of oxygen:
for example, at high elevations or during
prolonged physical exertion)

● Person with sickle-cell anemia
(severe sickling, usually dies in childhood)

Figure 34-2-1   Pedigree of sickle-cell anemia.

at such a high level while being selected against so strongly?

At this point we ask you to place yourself in the position of the working scientist. What explanations (hypotheses) can you think of that might answer the question?

This is not an easy task. We ask you to attempt this imaginative thinking so that you may become familiar with what is perhaps the most important job of the scientist. Technicians can often carry out experiments or make observations once it is clear what hypothesis should be tested. To develop a good hypothesis demands the knowledge and skill and talent of a scientist.

Scientists have considered at least three hypotheses to account for the commonness of sickle hemoglobin. One involves the mutation rate of the gene. The second pertains to differences in fertility. The third involves susceptibility and resistance to disease. Your job is to use these clues to devise three hypotheses that might explain why the sickle-cell trait persists in populations. (14), (15), (16).

After class discussion list the hypotheses, in

order for reference in answering the following questions.

It has been estimated by statistical techniques that the mutation rate necessary to replace the genes lost by selection would be about 100 times as high as the known rate for other human genes. There is no direct evidence of such a high rate for any human gene. Does this information support or weaken the first hypothesis? (17)

Present evidence indicates that individuals with the sickle-cell trait do not produce more children than do nonsicklers. Is our second hypothesis supported or weakened by this fact? (18)

The third hypothesis is a bit more difficult to investigate. As data on sickling were collected and the incidence of the trait was plotted on maps, it became clear that the gene was most common in a belt extending across the central portion of Africa. Malaria and hookworm are diseases that are very common in this region. Recall briefly from Chapter 1 some characteristics of malaria, including its symptoms, how it is transmitted, and how its symptoms are produced. (19)

Recall also that individuals with the sickle-cell trait have hemoglobin that differs from normal hemoglobin. Might the sickle-cell trait influence the incidence of malaria? (20)

This last question together with its answer really constitutes a new hypothesis. To test this hypothesis one investigator examined the blood of 290 children in an East African tribe where both malaria and sickling were common. The results are tabulated below.

	With Malaria	Without Malaria	Total
Sicklers	12 (27.9%)	31 (72.1%)	43
Normal	113 (45.7%)	134 (54.3%)	247

Do these data support the hypothesis? (21)

In order to test this hypothesis directly, 30 volunteers were inoculated with malarial parasites. The volunteers were adult males of approximately the same age and physical condition. A blood examination showed that none of the 30 had malarial parasites at the beginning of the experiment. Of the group, 15 had the sickle-cell trait and 15 had normal red blood cells. Two of the sicklers and 14 of the nonsicklers developed malaria. Do these results support the hypothesis developed in Question 20? (22) When the observations were completed, the individuals that developed malaria were given a drug to check the disease.

Answer the questions below, and explain your answers whenever appropriate.

Do the above observations show that, under certain conditions, the heterozygote may have a selective advantage over either homozygote? (23)

Do the observations indicate that a harmful mutant gene is necessarily harmful under all conditions? (24) Can a gene harmful under certain conditions be beneficial to a population under other conditions? (25)

Do the observations provide a clear example of inherited resistance to disease due to a single gene? (26)

The incidence of sickle-cell trait in the early American Negro population (whose ancestors, of course, were African) is estimated to have been about 22%.

In this country, man has virtually eliminated *Anopheles*, the malaria-carrying mosquito. Under these conditions, would the heterozygote have any survival advantage? (27) Recall that often the individual who is homozygous for sickling dies before reaching reproductive age. What do you predict will happen to the frequency of the allele for sickling in the United States? (28) Give a reason for your answer to Question 28? (29)

The incidence of sickle-cell trait in the present American Negro population is about 9%. Does this fact agree with your interpretation in Question 28? (30)

In this exercise has any evidence been presented indicating that a person heterozygous for the sickle gene is biologically handicapped insofar as professional, intellectual, or business endeavors are concerned? (31)

MAMMALIAN RADIATION

AND EVOLUTION

The chordate class Mammalia includes those vertebrates exhibiting their common heredity and ancestry by the following morphological characteristics:

1. The body is usually covered with hair.
2. The skull has two occipital condyles (points of joining with the neck) and there are usually seven neck vertebrae.
3. The nasal region is usually elongated and the teeth, rarely absent, usually occur in sockets. There is only one replacement of teeth during the life of the individual.
4. The tongue is usually mobile; the eyes have movable lids.
5. There are four limbs, and on each limb are five or fewer digits.
6. The heart is four-chambered.
7. Gas exchange between the organism and its environment is by lungs only; a complete muscular diaphragm separates the lungs and heart from the abdominal cavity.
8. The young are nourished after birth by milk secreted from mammary glands of the female.

Most of the mammals are found in two groups, the pouched or marsupial mammals (Metatheria), and the placental mammals (Eutheria). The placental mammals are subdivided further into such groups as mammals with nails (primates), gnawing mammals (two groups, the rodents such as rats, mice, squirrels, and beavers; and the hares and rabbits), carnivores, hoofed mammals (two groups, odd-toed and even-toed), cetaceans (the whales and porpoises that have returned to an aquatic life and lost their hindlimbs), and the flying mammals (bats).

The mammals arose from reptiles during the Jurassic period. By the early Cretaceous period they had advanced as far as the primitive

metatherians. Soon after, the subcontinent Australia, with its marsupial fauna, became isolated from other continents.

With the extinction of many of the dominant reptiles during the end of the Cretaceous period, ecological niches were ready for occupancy by the primitive mammalian forms. In Australia the marsupials, unhampered by competition with placental mammals, underwent adaptive radiation giving rise to many diverse forms as they filled these unoccupied niches in the environment. Elsewhere, however, the insect-eating mammals arising at the end of the Cretaceous period replaced the less fit and underwent rapid evolution at the dawn of the Tertiary period. The products of this evolution are visible today among the living placental mammals of the world.

It is a striking fact that environmental conditions have often greatly influenced the trend of evolution among the larger groups. A study of the mammals will provide illustrations of adaptive influences. Where different orders of mammals have met the same environmental conditions, they often show a convergence of adaptive characteristics. Where members of a single order of mammals have met differing environmental conditions, they often show a divergence of adaptive characteristics.

■ The purpose of this exercise is to study principles of adaptive radiation and convergent and divergent evolution by examining the orders of mammals.

MATERIALS: None

PROCEDURE

On a table that your teacher will give you, list along the left-hand margin a number of
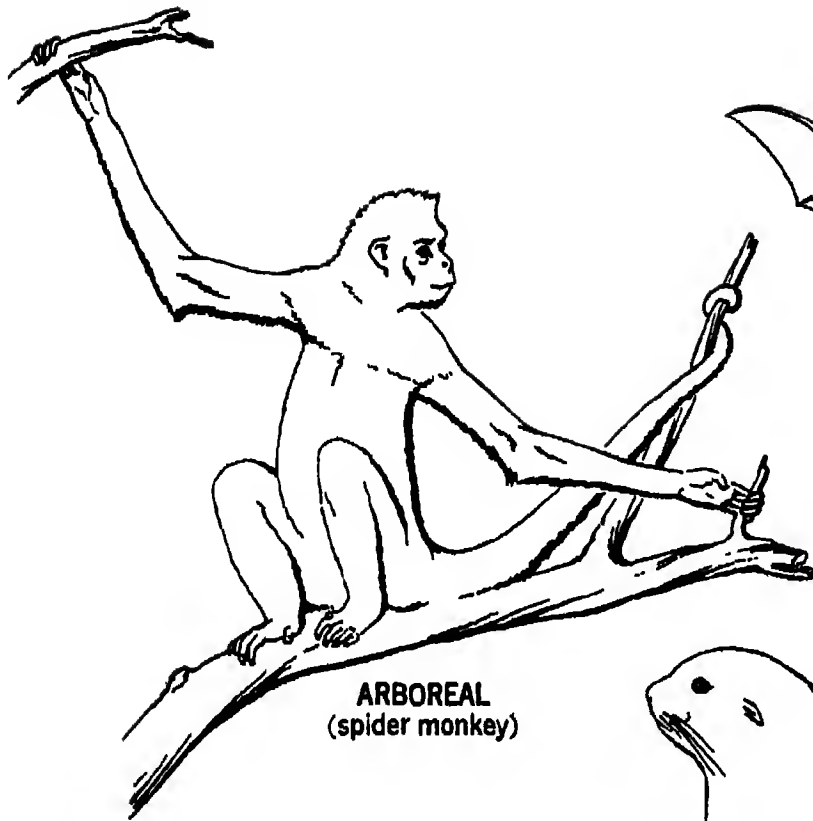
ARBOREAL
(spider monkey)

AERIAL
(bat)

AQUATIC
(fur seal)

FOSSORIAL
(star- nosed mole)

CURSORIAL
(horse)

Figure 36-1-1   Adaptations of five mammals to environmental conditions.
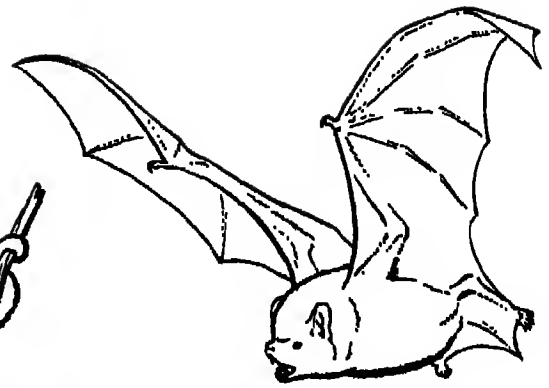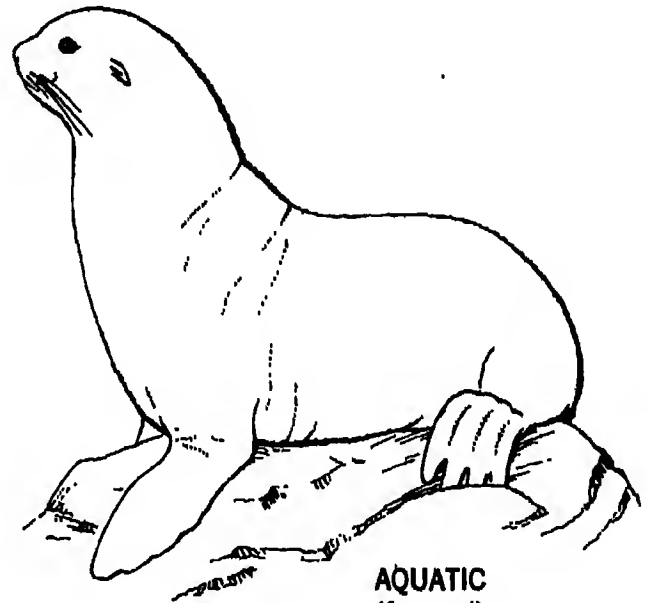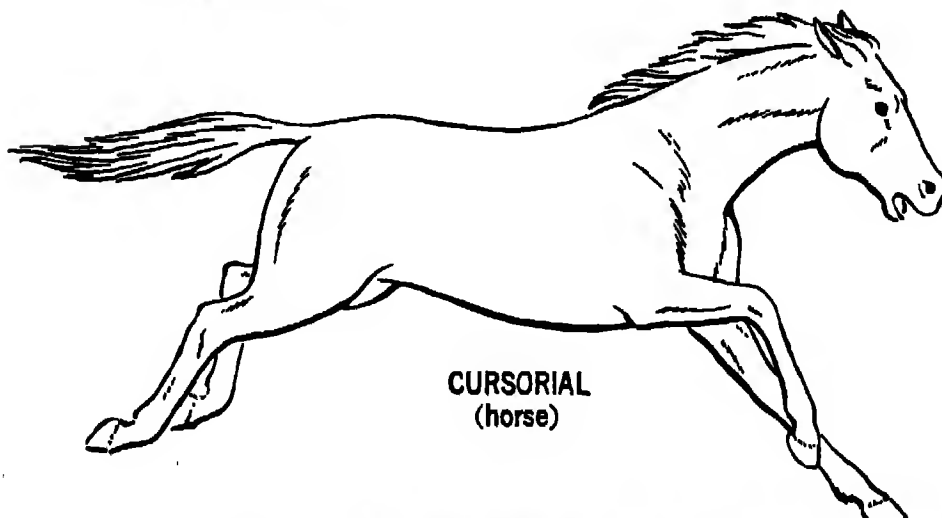
general types of adaptations of mammals to environmental conditions. Illustrations of some mammals and their adaptations are shown in Figure 36-1-1 and should be studied thoroughly before attempting to use the table.

Along the upper margin of the table, list groups of mammals and leave spaces to write in the names of mammals to be observed. Study each animal selected for observation, and record it by name or symbol in the proper column opposite each of the adaptations it shows. For example, you might use the symbols *L* for lion, *B* for bear, *K* for kangaroo, *O* for opossum, *Bv* for beaver, and so on. Make a list of all the symbols you use and their meanings.

When you have completed tabulating these data you should be able to note divergence within a group such as Metatheria simply by scanning down the appropriate column. By selecting any one adaptation and scanning across the table you will also see convergences between the groups. Using the table:

1. Compare the adaptive radiations of the Metatheria and Eutheria.
2. Discuss the most conspicuous examples of convergence. How do you account for these convergences?
3. Discuss the differences between parallel evolution, convergent evolution, and adaptive radiation. Cite examples of each among the mammals you have studied.

THE STUDY OF FOSSIL PLANTS

Throughout the chapters on plant and animal evolution there are references to the fossil record and what it tells us about organisms that lived long ago.

As you look through your textbook you will see illustrations and descriptions of plants and animals that grew millions of years ago. Many of these pictures and descriptions give very detailed information about the structure and reproduction of these extinct organisms. You may wonder how biologists know so much about the external form and even the internal structure of these ancient forms of life. A look at the various compression or impression fossils will show you immediately how we know what plants and animals of the past looked like.

■ The purpose of this exercise is to make you aware of the structural details that are preserved in some fossils and of the techniques that paleontologists can use to prepare fossils for study.

PROCEDURE

You will have either a fossil of a leaf or an ancient mollusk. The leaf represents only a small portion of a large organism while the fossil shell of the animal may be nearly complete. Thus, for most plants and many large animals,

especially vertebrates, the fossil remains of a whole organism may be fragmentary. It is the job of the paleontologist to look for clues which tell him which parts belong to one kind of organism and which do not. Can you think of some clues that would help in reconstructing whole organisms from their fossilized parts? (1) Obviously, to become a fossil, the plant or animal part must have become buried in sediments which later were changed into rock. What would happen to a plant leaf if it did not become buried in a place where oxygen was excluded? (2)

Examine the sediment in which the plant leaf was buried. Compare this with the sediment containing the mollusk shell. Put a drop of 10% HCl on sediments of both fossils. What is the reaction? (3) How do sediments in which these fossils occur differ? (4)

A "coal ball" is a kind of fossil in which petrified plant remains are preserved. The plant material retains some of its organic compounds even though it is embedded in a rock matrix. The matrix of a "coal ball" is usually calcium or magnesium carbonate, which has also impregnated the tissues of the plant. At the time the fossil was formed, this matrix of sediment —now rock—supported the plant tissues and kept them from being crushed. Although we can tell very little about the external form of plants from "coal balls," the "coal balls" do

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MATERIALS

Compression or impression fossils of a leaf

Fossil mollusk in limestone matrix

Precut calcified petrifactions of plants ("coal balls")

Carborundum grinding powder, No. 400

Plate glass 45 x 45 cm

10 ml of 10% hydrochloric acid (HCl)

2 liters of 5% HCl

Polyethylene squeeze bottle with attached pipette tip

1 liter of acetone

Roll of cellulose acetate film

Box 45 x 72 cm with 5-cm sides

Fine gravel or coarse sand to cover bottom of box with 4-cm layer

Razor blade

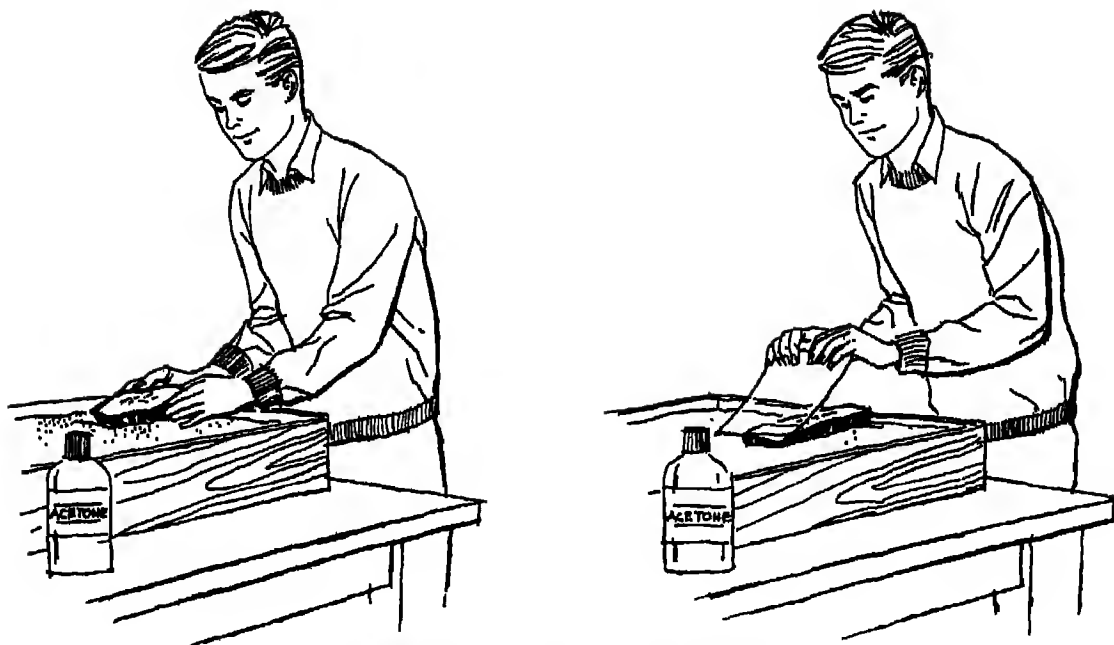Stereoscopic dissecting microscope or compound microscope

Figure 36-2-1 "Coal-ball" technique.

give us detailed information about the internal structure of the plants. To get this information we must prepare sections of the "coal ball" that can be examined under a dissecting microscope or a compound microscope.

Polishing the cut surface of the "coal ball" is accomplished on the glass plate by using a little No. 400 carborundum powder and a few drops of water. Simply rub the cut surface of the specimen in the wet carborundum until the surface of the specimen is smooth to the touch. Wash the polished surface with water. Be sure to remove all of the carborundum powder.

Etching the polished surface of the specimen is done by placing the polished surface in 5% HCl for approximately 15 to 20 seconds. The coal ball will effervesce during this procedure. (CAUTION: *5% HCl will not injure the human skin but it can ruin clothing if it is spilled. Use great care while doing this part of the exercise.*) What part of the "coal ball" is the acid attacking? (5)

Immediately after the etching is completed the etched surface of the "coal ball" should be washed under the tap with water running slowly. *After this step is completed, great care must be taken not to touch the etched surface.* If you touch the surface you will break the delicate cell walls of the plant fossil which now project from the etched surface of the "coal ball."

Drying the etched surface is accomplished by placing the "coal ball" in the box of gravel, etched surface up. Within a few minutes the surface will turn white as it dries in the air. If you are pressed for time, the surface can be flooded with acetone from the polyethylene squeeze bottle. Repeat the flooding two or three times or until all traces of water disappear from the surface.

Making the "peel" comes next. Orient the "coal ball" in the box containing gravel or sand so the prepared surface is slightly tilted.

Cut a piece of acetate film slightly larger than the prepared surface of the specimen. Flood the surface of the specimen with acetone. Most of the acetone will accumulate along the lower edge of the "coal ball." *Quickly* lay the acetate film in the acetone along the lower edge. Lower the film onto the surface of the "coal ball" so that the acetone is pushed ahead of the film toward the higher edge (see Figure 36-2-1) so as to make a uniform layer of acetone between the film and the surface of the specimen.

Now let the acetate–acetone "peel" dry for 20 minutes. The surface with the "peel" on it will turn whitish as drying proceeds.

Removing the Peel. After it has dried, the peel can be removed by taking one edge in your thumb and forefinger and carefully pulling the peel from the surface. If it starts to tear, use a razor blade to loosen it at the point of the tear.

Put the peel on the stage of a dissecting microscope, using a white background on the stage. What parts of plants are preserved in a "coal ball"? (6) How does this help us understand the structure of the plants of the past? (7)

Ecology

This topic is deliberately placed at the end of the laboratory book for two reasons. First, wherever you may be geographically, the weather should be pleasant enough to allow field work in the spring. Second, these exercises should challenge you to use the knowledge gained from previous work in the laboratory and from the textbook to analyze not simply one living organism, but communities of living organisms in relation to one another and to their environment—a new dimension of life at the population and community level.

It is sometimes difficult in laboratory studies to remember that an organism does not live isolated in a test tube or in a laboratory cage, but lives rather in a complex natural environment with varying kinds of food, with varying temperatures and humidities, with different kinds of plants and animals as associates, and with all the dangers and satisfactions that come from associating freely with both similar and different organisms.

Our field work will allow us to see living terrestrial communities in which we can identify many different kinds of plants and animals and compare their distribution and numbers in light of the relationships they bear to one another.

We will also have the opportunity to notice the phenomenon of succession and to observe that both the living and the nonliving portions of the world do not remain the same but undergo definite and predictable cyclic changes characteristic of each environment.

The opportunity to study living organisms in their natural environments and to see the broad picture of the interrelationships of a physical environment with its microorganisms, plants, and animals should prove to be the crowning achievement of your biology course.

A PLANT COMMUNITY

A terrestrial community includes all living things within its boundaries. But it is more than just a collection of plants and animals. Mutual interrelationships exist among them—both competitive and cooperative. Each organism is dependent on others and on the physical environment in which it lives.

Plants are usually the most conspicuous components of the terrestrial community. When we use such terms as "oak ridge," "hickory flat," "gum bottom," "cypress swamp," "grassy meadow," or "stand of pine," we name communities and, to a limited extent, we describe them. However, a more complete description of any community requires that many additional specific and general characters be observed and stated. In this exercise we will attempt to define more accurately a community on the basis of its plants.

■ The purpose of this exercise is to study the interrelationships of plants in a nearby field or forest. We will measure the coverage (area of occupancy), and abundance of the more common organisms and calculate their densities. We will then attempt to evaluate their functional relations in the community.

MATERIALS

The specific materials needed will depend upon the methods you use, but the following list is minimal for the field.

Twine (over 100 m long)

Four stakes

Hammer

Compass, magnetic

Millimeter ruler (6-cm)

Notebook, paper, pencil

Graph paper

Two meter sticks

Hoop of 1-sq-m area

Plastic bags

Tin can, 5-cm diameter

PROCEDURE

Your teacher will have assigned you to a team or paired you with another student. For any collections you make, you will be responsible to your team for marking properly the (a) exact location, sample number, and date of study; (b) names of the team members and the team number; and (c) specific kind of sample taken. Your teacher will ask you to reserve certain samples for later study. Each student will take notes in the field. *It cannot be overemphasized that without proper record-keeping the entire exercise will be fruitless.*

Selecting and Plotting the Site

Select the sample site and set up the boundaries of the square sample area (quadrat) as follows:

Measure a 100-m length of twine. Make 0.5 m divisions of the 100-m length by tying colored twine, or by tying knots at each 0.5 m mark. If you use the knot method, the final 100 m should be determined *after* all the knots are made.

Drive a stake at the edge of the site. Using a compass, sight the opposite corner. Drive the second stake exactly 70.7 m from the first stake (Figure 39-1-1A).

Tie one end of the 100-m twine to each stake. Then pick up the center mark on the twine and walk at right angles to an imaginary line between the first two stakes. Drive the third stake at the point where the line is tight (Figure 39-1-1B). Reverse the process to place the fourth stake (Figure 39-1-1C). This will give a 50-m-square site for examination (Figure 39-1-1D). If you live in an area heavily populated with plants or animals, smaller plots may be used. *Exercise care to disturb the enclosed area as little as possible.*

Plot the site on graph paper and arbitrarily divide it into subplots. There should be a minimum of nine subplots (see Figure 39-1-2).

Now stand back and look at the site. If necessary, walk around the outer boundary. Look for the following features.
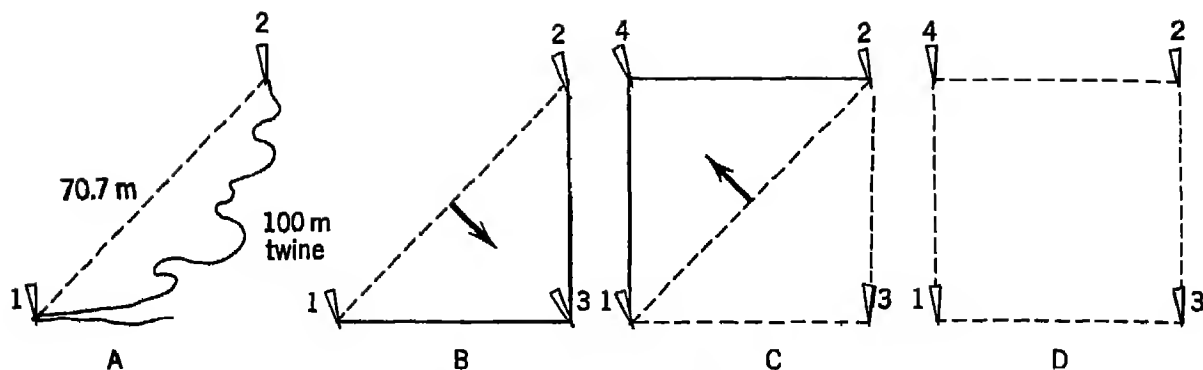
Figure 39-1-1 Staking out the site.

Trees. Trees make up the canopy layer of a community. The canopy layer is the top layer or ceiling. There may be smaller trees below the tallest which make up the sub-canopy layers.

Shrubs and Saplings. Shrubs may be defined as low woody plants measuring between 0.5 m and 3 m in height. A sapling is a young tree less than 4 and more than 2.5 cm in diameter at chest height (1.35 m).

Herbaceous Plants and Tree Seedlings. An herbaceous plant is any nonwoody plant which dies down to the ground at winter. These include the grasses, grains, and small flowering plants, including those we normally call weeds.

Floor Covering. The floor covering is made up of the litter (fallen leaves, stems, etc.) and certain flat plants such as mosses and lichens.

Site Profile

Using the following legend, draw a profile for the community as you understand it from the general observations. Your profile should follow the pattern shown in Figure 39-1-3.

Layers
 Canopy Layer (Minimum 20 m tall): ◯
 Subcanopy Layer (Maximum 20 m tall): ⋏
 Shrubs (1–3 m): ○
 Herbs: △
 Floor Covering (litter): ▬

Plant Types

Woody Vegetation		Herbaceous Vegetation	
Deciduous	D	Grass	G
Evergreen	E	Nongrass	N

Indicate with numbers the various plant types that are recognizably different (species) as shown in the profile drawing.

Once the profile of the site is made, we are ready for a closer look at its vegetation and are ready to measure cover, density, and abundance. It is advantageous for one pair of students to examine one portion of the plant community; one pair for trees, one pair for shrubs,
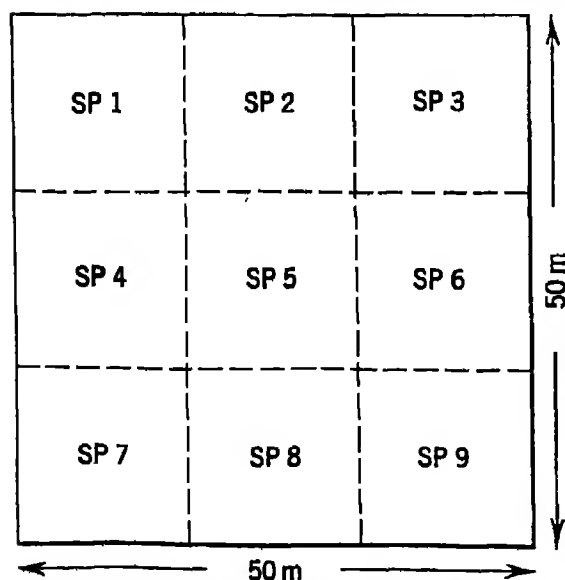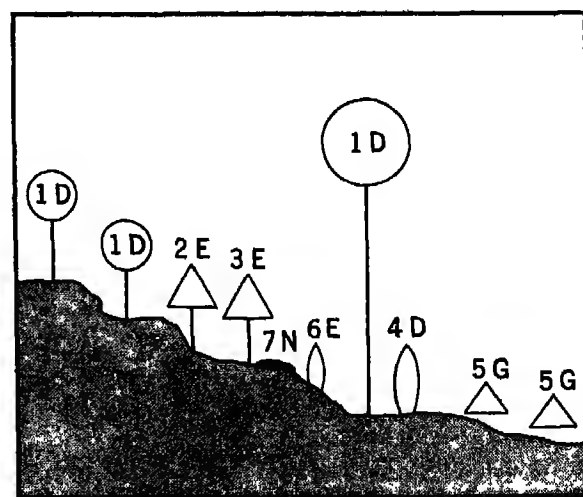

Figure 39-1-2 Dividing the site into nine subplots.

Figure 39-1-3 Example of a profile.

one pair for herbs, and one pair for floor covering.

Cover

Cover is the term generally used to indicate the area occupied by the species and is usually a measure of the area covered by the crown (trees), the stem (shrubs and herbs), or the litter.

Trees. If your site or plot is sparsely wooded, measure the diameter of the crown of each tree found there by estimating on the ground the distance the branches extend from the trunk. If the site is heavily wooded, use the meter stick method of sampling. Record which method you use. In the meter stick method, one student holds two meter sticks, one in each hand at right angles to the body at chest height (about 1.35 m). The student then walks a 20-m transection of the wooded area. All trees, large or small, that are touched by any part of either meter stick are used in the survey and their diameters are measured and recorded in chart form according to the following instructions. This survey usually takes three persons, the "meter stick" person, the measurer, and the recorder.

Regardless of the site, make a chart with the species number recorded in a column down the left side (add names if you know them). Record the diameter sizes along the top: for example, you may separate stem diameters into 10-20, 21-30, 31-40, 41-50 cm, etc. The crown diameter, may be classified 1-6, 7-12, 13-18, 19-24 m, etc., by estimating on the ground the distance the branches extend from the trunk. In the body of the table list the number of trees of each kind under their appropriate sizes. After your table is completed, notice which species contain the largest trees and whether certain species appear to be reproducing their kind. For example, if tree species No. 1 has 70 individuals in the largest size class and 10 in the smallest, whereas species No. 2 has 40 in the largest class and 50 in the smallest, it is possible that species No. 2 is reproducing its kind faster than species No. 1 and may eventually replace species No. 1.

The diameters of the tree trunks can also be used to find the cross-sectional area of the trunks, or the basal area of the tree. Substitute the diameters of the trunks for d in the formula $\frac{\pi d^2}{4}$. This is the standard formula for calculating the area of a circle from the diameter; $\pi = 3.1416$. Take the middle number in each size class to represent the average diameter

of all trees in that class. Calculate its basal area and then multiply this basal area by the number of trees in this category to give you a figure representing total cover. The basal area serves as a measure of the relative importance of the various trees, since it takes into account not only the number of trees but also their sizes.

Shrubs and Herbs. Choose plots 1 m square within the site in which to study the shrubs and herbs. This may be done by throwing a stick or coin at random within the site or by sampling from *each* subplot of the site. A second acceptable method is to throw or to lay a hoop whose area is 1 sq m. (If others working with you are collecting insects it is best to *lay* the hoop to avoid scaring away insects.)

The instructor may designate certain prominent species to be studied as cover or may divide the vegetation into categories such as tall grass, short grass, or herbs. Your instructor may ask that certain plants be preserved and will instruct you in ways of preserving them.

Two methods are available for measuring the cover of shrubs and herbs.

1. The linear method: Extend a line for 50 cm through the area. Measure the length of the line that is in contact with shrub or herb cover. To determine the shrub or herb cover, or space occupied by shrubs, convert linear measurements to a percentage basis. For example, if a shrub species extended along 15 cm of a total sample line of 50 cm, the cover value for this shrub would be 30% $\left(\frac{15}{50} = 30\%\right)$.

2. Diameter and area method: Select 25 stems of each of the species you are to collect and measure their diameters. Total the class data for diameters of each species and divide by the number of stems measured. This will give you an average stem diameter for each species. Multiply the number of individuals of each species per square meter by its average stem diameter to obtain the cover of each species per square meter.

Make a list of all the species with their areas of coverage in order from the greatest coverage down to the smallest coverage.

Abundance and Density

Abundance. Abundance refers to the numbers of plants belonging to an individual species within a given area. Abundance is important when trying to determine the relative importance of a species in a community. The abundance is related to density, the number of individuals in the unit area sampled. To determine abundance or density, plots of the

following sizes are recommended for plants of each size.

1. Trees—a 10-sq-m area
2. Shrubs or medium-sized vegetation—a 4-sq-m area
3. Low vegetation (herbs)—1-sq-m area

Each pair of students is to count the plants in the particular squares assigned by the instructor. Make a list of the different plants to be counted in the given area. Across the top of the table list each sample plot in separate columns. Within the table record the number of stems of each species in each sample plot. After all the data have been recorded, compute the average number of each species as a measure of its abundance.

Make a list of the plants from the most abundant to the least abundant species.

Density. Once the abundance of a species or plant has been computed, the determination of density is easy. Simply add the total number of each species for all the sample areas and divide the sum by the total area in square meters:

$$\frac{\text{total number of each species}}{\text{total sample area in square meters}} = \text{density}$$

Make a list of the plants from the most dense to the least dense species.

Density may be estimated at the same time as the initial survey is made by using the following guide:

Density

c = continuous growth
i = plants usually do not touch
p = woody plants scattered singly or in groves; herbs in disconnected patches
r = rare but conspicuous
b = barren; vegetation largely or entirely missing

Litter and Soil Cover

From the ground around each of the following portions of the plant community—shrubs, herbs, and litter—collect a sample of litter and soil cover to be taken back to the school laboratory for study. Ask your teacher for preservation methods.

Each student in your group is to examine a 15-cm-square (225 sq cm) sample of litter and soil cover in place. Carefully pick the sample apart and record the layers and composition of each layer. Also record its physical state: state of decay, H_2O level (damp, moist, dry), odor, location as to shade, and proximity to other plants.

For all 15-cm-square samples examined, summarize the number of layers and their composition. Calculate the density.

Make a brief group summary of the important features that characterize the plant community you have studied including profile, cover, abundance, density, and litter and soil cover as they apply to the community studied by your team.

The summary reports of each team will be duplicated and distributed to each member of the class for analysis of the study area by the class as a whole.

Communities are usually named according to the most important species of plants. For example, a deciduous forest might be called a beech-maple forest if these trees were dominant. Dominance in your study might be decided on the basis of greatest (a) density, (b) basal area or numbers in the larger size classes of diameters, (c) percentage of cover, or some combination of these features. After you have carefully analyzed the data, determine the one or two species that appear to be most important. (1) What will you name this community? (2) How many layers of vegetation did you find in the entire study area? (3) List the important species in each of these layers. (4) Some of the plants are in a layer because they are limited genetically to that maximum height, while others are simply young individuals that will some day reach higher in the community. Examine the plant data from the herb, shrub, or small tree layers and list the plants in the latter category. (5) If you were to return to this plot in 1000 years, what tree would you expect to be dominant if climatic conditions remain the same? (6) If time allows, you may wish to investigate other plant communities in your vicinity to determine their composition.

AN ANIMAL COMMUNITY

In Exercise 39-1, we studied a plant community and the relationships of various plants to one another. However, during our study of the plant community, insects and birds were seen and perhaps other animals as well. It becomes quite obvious that there is really no such thing as a plant community or an animal community existing independently of each other. Therefore, while it is convenient to separate the animal and plant communities for purposes of studying them, in reality they are closely related and dependent upon each other. While doing this exercise on animals and their community relationships, keep in mind the essential plant community in which the animals are found.

■ The purpose of this exercise is to study the interrelationships of animals existing in a plant community. We will determine the location of various animals within the community and their relative abundance and density. We will correlate these features of the animal community with the interrelationships between the animals studied and the plant community in which they exist.

PROCEDURE

This field study concerns the animals and their functions in a community. There are four parts to the field collection and teams will be assigned to each part. The four collections are: (1) soil samples to be taken to the laboratory where the very small animals will be removed; (2) the larger animals from the soil surface in the field; (3) the flying insects and related invertebrates; (4) observation, and if feasible, collection of the vertebrates.

The site for these studies of animals should, of course, be the same as that studied for plants in Exercise 39-1 so the relationships of all the organisms of the community can be studied. If you do not have a site plotted for study, refer to the section of Exercise 39-1 on Selecting and Plotting the Site for instructions on setting up a study area.

Collecting Microscopic Soil Animals

This step in our community study is to collect soil and leaf litter samples from which we can extract minute animals. One team may collect the layer of undecayed plant leaves from a quadrat 25 cm square. These leaves should

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MATERIALS
(if study site not already established):

100 m or more of twine
Stakes
Hammer
Compass, magnetic

Field Materials
Notebook, paper, pencil
Ruler, 6-cm
Graph paper
Two meter sticks
Hoop of 1-sq-m area
Plastic bags
Tin can, 5-cm diameter
Sweep net

Preservative in labeled bottles, 70 % ethyl alcohol or another
Cyanide bottle or dropper bottle of carbon tetrachloride, lighter fluid, or 3 : 1 mixture of gasoline and chloroform

Laboratory Materials
Funnel
5-8% formalin in container or 70% ethyl alcohol
Steel wool
Light source (gooseneck lamp)
Cellophane tape
Compound or stereoscopic dissecting microscope
Wax pencil

be *sealed* in a small plastic bag for transportation to the laboratory. Each bag should be labeled with the name of team, name of specimen type, date, and subplot from which taken.

After removing the undecayed leaf litter from the plot, cut a core sample of the soil down to a depth of 8–10 cm. This core can be conveniently taken by using an orange juice can of about 5-cm diameter. Be sure to seal this sample in a plastic bag and label it. Take at least two soil samples from each plant area.

When you return to the laboratory, place each sample in a funnel containing a small piece of steel wool, with the neck of the funnel suspended just above a small container of 5% formalin. The steel wool in the bottom of the funnel will hold back the litter and soil but will allow small organisms to pass through. Above the funnel a small light bulb should be fixed several inches from the sample material. This light and heat, with the subsequent evaporation of moisture from the soil, forces many of the organisms in the samples to move downward into the preservative. This method is especially satisfactory for the collection of mites and small insects such as springtails. It should be noticed that after the soils have been heated for about 7 days there is little likelihood that any more animals will be found for several weeks. This apparatus for collecting small organisms is called a modified Berlesi funnel; your teacher will discuss this with you.

Once the animals have been collected from the soil sample, examine them under low power of the compound microscope or with a stereoscopic dissecting microscope. Record the species of animals by name or by drawings. Count the number of each species present, and record each in a column opposite the particular soil specimen from which it came. Total the number of each species of animal found in all samples.

Record the totals of each species in the soil samples in descending order of abundance (from the most abundant to the least abundant). From the data above compute density.

$$\frac{\text{total number of each species}}{\text{total sample area in square meters}} = \text{density}$$

Collecting Macroscopic Soil Animals

Another procedure in our habitat analysis is to capture and count the macroscopic animals in the soil and leaf litter. A convenient sample marker for your area can be made from a clothes hanger bent to form a circle, a used

bicycle rim with the spokes removed, or a hoop. Any of these will give a convenient, standard boundary for the sample area. The marker is laid down at random or can be placed in specific areas in the habitat that you wish to study. The students from one team then capture and preserve *all* animals that they see within the enclosure. Two samples can be taken within each hoop: 1. the animals found in the organic materials (litter) on the surface of the soil, and, 2. the animals captured in the soil (say from 0–10 cm depth). These samples should be placed in bottles and plainly marked with a wax pencil as to the *kind* of sample, the date, the *location*, and the team.

Since many of the macroscopic animals will fall into the insect group, refer to the section, Collecting Flying Insects, later in this exercise for additional suggestions for trapping.

Make careful observations of each plot for spider burrows, etc.

In the laboratory, sort the animals of each sample into groups of obviously different species of animals. List each species by name or number. Count the number of each species and record it opposite the name of the species in a column bearing the sample number. Total the number of each species found in all samples.

In your notebook record in a list the species totals of macroscopic soil animals from the most abundant to the least abundant.

From the above data, compute the density as you did for the microscopic soil animals.

Collecting Animals Living on Plants

Many animals can be found only on plants. Using random sampling or a specific plot, examine a random assortment of the plants, looking particularly on the undersides of leaves, at the apex of the leaf and stem, on the flower buds, and on the growing tips. Pick off the leaf, the stem, *or* the bud if they bear evidence of animal life, and drop the entire specimen into a container of formaldehyde, 70% alcohol, or other preservative.

Label with type of collection, date, exact location, plant type, and team number.

In the laboratory make a chart in your notebook and record each plant type, plant part, species of animal living on the plant, and numbers of each species. List them in order, the most abundant at the top. Compute density as before.

Collecting Flying Insects

A collection of flying insects can be made by using an insect sweep net. Make 48 low

sweeps over the vegetation with this net, one sweep with every step you take in a straight line. Brush the vegetation gently as you make your sweep with the net. This sample may be considered roughly equal to a covering of 1 sq m. Quickly close the net and place it inside a large plastic bag into which you pour a few drops of 3:1 mixture of gasoline and chloroform. (DANGER: EXPLOSIVE!) After a few minutes the insects in the net can be sorted and all of them placed into a jar of preservative. Label each jar with type of collection, date, area covered, and team number. Alternately, the usual cyanide bottle can be used but with *extreme caution* since its fumes are deadly. After the insects have been killed, they can be sorted and put into labeled jars of 70% ethyl alcohol or 5–8% formalin.

In the laboratory, sort the insects into groups of obviously different species. List each species by name or number. Count the number of each species and record opposite the species name in a column bearing the sample number. Total the number of each species found in all samples in descending order of abundance (from the most abundant to the least abundant). From this data compute the density as you did previously.

Collecting and Observing Vertebrates

Amphibians and Reptiles. The vertebrates of a habitat are the more difficult organisms to study. Turning over rocks, logs, or metal objects on the surface of the soil while you walk through an area may yield a collection of some amphibians and reptiles. If you move logs or stones, be certain to return them to their original positions, so that you do not ruin the habitat for the organisms that live there. Collect and preserve the specimens as directed by your teacher.

Record the total number of amphibians and reptiles in order of descending abundance for each of the habitats examined.

Birds. For most practical purposes, birds must be studied by observations — sight and sound. It is possible for particularly interested people to become associated with a local, authorized bird-watching or bird-banding club or maybe the club itself will become interested in your program. Your teacher may ask some students on weekends and after school to set up bird blinds to observe nests. Binoculars are necessary for the best observation and identification, preferably 6 × 30 or 7 × 50. Bird tracks, eggs, and nests also offer a means of observing the presence and range of a species.

Records of bird songs are a good aid to identification. Flight patterns, food habits, locations, and particular behavior patterns also are helpful and should be included in your observations. The number of birds of different kinds heard and seen on a walk of a given length, direction, and time will help to standardize your concepts of abundance and range.

Record in chart form similar to the others you have made the data collected and calculate the abundance and density of birds.

Mammals. Mammals and other vertebrates can be directly observed and collected, or indirectly assumed to be present because of their traces, tracks, remains, sounds, or homes. It is more difficult to determine abundance and cover for mammals because they move about a lot and therefore no very precise directions as to the placement, size, shape, or number of sample plots can be made.

Abundance refers to the *number* of individuals present. For plants, abundance is obtained simply by counting individuals either in the whole area or in sample plots. For moving mammals, sample plots can be used to determine abundance indirectly by counting traces, tracks, or homes of the various mammals in the sample plots. Live-trapping and marking individual mammals so the same one will not be counted twice is another method of obtaining data on their abundance. Observations of unmarked animals is of some value but for rough estimates only.

Record the numbers and species of mammals in order of abundance. Indicate the density of each species in the study area.

Class Analysis of Animal Communities

When you have finished your animal collecting trip, you will have collections of animals from the soil surface (litter), the mineral soil beneath the surface, of flying insects on the low vegetation, and of vertebrates.

All summary charts will be duplicated and distributed to the entire class for analysis.

You will have separated each species of animal from others. The number of each will have been counted and recorded. You will have recorded the place of collection, the density of each group, and their abundance.

Make a chart recording the density of the different species of animals located around the various types of vegetation (trees, shrubs, herbs, litter) of the plant community. On the basis of these calculations, which layer of the community has the greatest abundance of individuals? (1)

Arrange the animals in order of those occurring in least numbers to those occurring in greatest numbers. (Example: bears 1, cows 6, etc.) What general trend is shown from this arrangement? (2)

All of the green plants are food producers. Groups of animals such as the leafhoppers, aphids, grasshoppers, moths and butterflies, and most mice and deer are called first-order consumers (herbivores). The beetles (certain ones), spiders, centipedes, salamanders, and shrews are second-order consumers (carnivores). Others such as fungi (plant), mites, springtails, isopods, millipedes, snails, earthworms, and crickets are decomposers or feeders on dead organic materials. Many groups are not easily catalogued in this food chain or food web since they include species having variable food habits (omnivores).

Make a list of each species of animal and what it eats. (3) After you have made this list, determine how many are first-order consumers. (4) How many are second-order consumers? (5) List examples in which one animal eats another animal, which in turn eats a plant. (6)

Diagram the food relationships (food web) of your community. (7) What animals known to occur in the area are missing from your collections? (8) In your data which animals are more numerous; the first-order or the second-order consumers? (9) Are there more first-order or second-order consumers in any particular layer? (10)

After having considered *all* the data gathered in this exercise, write your conclusions about the interrelationships of the different populations in this community.

ECOLOGICAL SUCCESSION

Most communities of plants and animals are always changing. One group of organisms is replaced by a second; the second is later replaced by a third; and so on through a succession of distinct population changes. In some instances familiar fields and woodlands may change greatly in appearance in a few years. In other instances a century or more may be required to bring about a noticeable difference. As plants of the community change in kinds and numbers, a corresponding change usually takes place in the insects, birds, mammals, and other animals in it. These changes are accompanied by increased complexity of food relationships.

An interesting thing about these changes is that they all seem to move in one direction. If left undisturbed, the steps in successional changes do not retrace themselves; neither do they continue forever. Instead, a stable (climax) community results as a final stage in the series of changes. This climax community perpetuates itself as a delicately balanced condition of active or dynamic equilibrium. As rapidly as plants and animals of the community die, they are replaced by others of the same kind. Although there may be minor and periodic changes in the population of certain species, the community as a whole continues to maintain the characteristics of the major type of biome of the area.

■ The purpose of this exercise is to observe different stages in plant and animal succession in two contrasting plant communities, one in a very wet habitat and the other in a dry habitat. You will also have occasion to observe stages in a series of changes taking place in the establishment of a plant community characteristic of a particular climate.

PROCEDURE

Part A:
Plant Succession
Starting with Open Water

Visit a pond selected in advance by your teacher. Notice that the plants are grouped in more or less concentric communities around the margin of the pond.

Ponds vary greatly in size, shape, and in the kinds of plants and animals present. The pond selected for your study may lack one or more of the stages described in the following paragraphs. Missing stages may have been present earlier or they may become established at some later time.

It is difficult to realize, as you look at a pond, that it is slowly disappearing. While the rate of disappearance is hardly a matter of "here today and gone tomorrow," all ponds are, in a sense, temporary. Soil is washed in and settles to the bottom. Decaying plant and animal matter accumulates to build up the bottom still further. Deep water eventually becomes shallow; shallow water becomes swamp and finally dry land.

Each pond community you observe, then, represents one of six or more successive stages

MATERIALS

Field notebook
Ruler at least 20 cm long
Pencil
Small boat

Wading boots
Weighted line equipped with hooks
Digging equipment (mattocks and shovels)

in the disappearance of the pond. Observe the communities as viewed from the shore of the pond. If a boat is available, some members of the class may be able to gather samples of plants growing in comparatively deep water with hooks attached to the end of a long, weighted line. These lines may be used as casting lines to collect submerged plants in deep water if a boat is not available.

Observe and list all of the communities found in and around the pond. (1) Which, if any, of the following communities described are missing from your pond? (2) These communities are composed of both plants and animals, but they are named for the more conspicuous plants present. Beginning with the plants growing in deeper water and progressing shoreward, the communities usually present are those described in the following paragraphs. Observe carefully and attempt to see how each community is related to others of the series. No attempt will be made in this exercise to collect and identify all or most of the plants and animals. Attention will be focused instead on communities and their interrelationships.

Submerged Aquatic Plants. Plant members of this community are entirely submerged algae and seed plants which grow at depths ranging from a few centimeters to 5 m or more. Deep water in the middle of the pond may have only plankton algae. Animals present include fish, crustaceans, insects, and microscopic forms. Because they are more freely mobile, animals are not as sharply restricted to certain habitats as are plants.

Use a weighted line provided with hooks to obtain samples of submerged aquatic plants from the deeper water of the pond beyond the edge of the zone of floating aquatics. Notice the leaves and other vegetative parts of these plants. Describe these vegetative parts. (3) Can you suggest any advantage these leaf shapes might have over others? (4) Describe any animals you might find attached to the plants. (5) As their remains accumulate after death and decay, plants and animals of the submerged aquatic community gradually build up the bottom of the pond. Would you expect the accumulation of organic and mineral materials to take place more rapidly in deep water or in shallow water? (6) What effect might this difference in rate of accumulation of materials have on the general shape of the bottom of the pond? (7)

Aquatic Plants with Floating Leaves. These are commonly called floating aquatics, al-

though only the leaves and flowers float. They are usually rooted in the soil at the bottom. Fleshy stems of water lilies and other broad-leaved plants of this community push their way through the mud of the bottom to spread vegetatively over large areas. The depth of the pond itself prevents their spreading to deeper water.

If you have wading boots, examine the bottom beneath the closely crowded leaves. List any animals you may recognize. (8) Compare the abundance of submerged aquatics on the bottom beneath the floating leaves with those found in the open water. Explain. (9) Compared with the open water, can you suggest any advantages the floating aquatic community might have for animal life? (10)

The floating aquatic stage, like other communities of the series, builds up the bottom soil still further. In doing so, it brings about its own passage from the scene and prepares the way for the next community. What really happens, at least for many years, is that the concentrically arranged plant communities do not disappear; they merely move. Which way? Explain. (11)

Emergent Aquatic Plants. As the bottom of the pond gradually rises higher, cattails, rushes, and other upright plants of this community invade, rise above, and overtop the floating aquatics. In the resulting competition for light, space, and soil, the floating aquatics eventually become loose and are replaced. As sediments and organic matter accumulate still further, the emergent aquatic stage is gradually replaced by a sedge-grass community growing in saturated soil or water only a few centimeters deep.

Sedge-grass Community. This stage is often called "swamp grass." It may form a narrow band of vegetation around the pond or it may occupy poorly drained areas of many acres. Tall sedges and grasses of this community form the natural home of many aquatic birds and other animals. List any of these which you may see and recognize.

Shrub Community. Willows, buttonbush, alder, and other shrubs may invade and replace the sedge-grass community or they may follow directly after the floating aquatic community. A trench dug at right angles to the shore for a distance of 2 to 3 m from the shore may uncover at progressively deeper levels partly decayed remains of earlier stages in succession. If you can get permission from the owner and if time permits, dig such a trench. Examine and record your findings.

Tree Climax or Grassland Climax Community. Depending on the climate of the region of your pond, the higher ground behind the shrub stage normally supports either a climax forest or grassland. Which type of climatic climax occurs in your region? Explain. (12) If left undisturbed, the climax vegetation and the animals associated with it will live in an active but balanced state of equilibrium indefinitely. Fire, timber cutting, or other activities of man may cause the successional picture to return to the sedge-grass or shrub stage, after which the successional pattern would again repeat itself.

A Modification of the Usual Succession. A special modification of the sequence of communities occurs in certain glaciated regions of the northern United States and Canada. If the pond you are observing happens to be in this region, you may find a sedge mat of densely tangled roots, rhizomes, peat, and assorted debris bordering the open water and floating upon it. Be careful in your examination of a mat of this type. You may break through.

Bog shrubs and bog trees usually invade the sedge mat near shore. As the soil is built up still further, a climax forest is eventually established.

The total time required for the completion of all stages leading to the disappearance of a pond or lake and the subsequent establishment of a climax community may vary from a few decades to many centuries. Can you think of any conditions or factors that might influence the length of time required? (13)

In the various stages of plant succession which you have observed there are reasons why each kind of community is replaced by the one which follows it. List in order in a column the communities observed. After the name of each community list at least two reasons why it was replaced by the next community. (14) Why is the climax community not usually replaced by another community? (15) Under what circumstances might the climax community be replaced by another community? (16)

Part B:
Plant Succession
Starting on Bare Rock

Plant succession starting on bare rock is like that starting in open water, in that both have definite stages represented by different plant communities. A second point of similarity is that both types of succession may be expected to produce eventually the same type of climax community.

The area selected for this part of the study is a rock outcrop, boulder field, or any location where large areas of bare rock have been exposed to weathering for several years. The communities to be studied will show succession from very dry to intermediate conditions. Different communities are not as distinct as they were in the pond nor will they be arranged in such an exact order.

Crustose Lichen Community. Pioneer plants that first become established on bare rock are crustose lichens and the moss *Grimmia.* Locate a rock surface with crustose lichens upon it. Observe the surface carefully for these lichens may look like gray, black, green, or orange discolorations. Particles of the underlying rock are slowly loosened and broken down by the mechanical and chemical actions of the lichens and *Grimmia. Grimmia,* if present will appear as shallow cushions of compact, almost black moss, when dry. When wet it is dark green. Rock particles, partially decomposed lichens, and trapped dust particles accumulate to form the first thin layer of soil. The soil then tends to collect in pockets and crevices. This plant community may persist for many years before it is replaced by the next stage.

Foliose and Fruticose Lichen Stage. As soil is accumulated by the crustose lichen stage, larger, leaflike or upright, branched lichens move in and become established. Reindeer moss is one of the more common of the fruticose lichens. Most species resemble very small, light gray, much-branched, leafless trees. With a small ruler measure the depth of the soil beneath two or three communities of foliose and fruticose lichens. Record in your notebook. Compare the depth of soil with that beneath the crustose lichen community. (17) Can you suggest any reasons why the foliose and fruticose lichens crowd out the crustose lichens? (18)

The Moss Community. With the exception of *Grimmia,* other mosses, such as the common pigeon wheat moss, and clubmosses such as *Selaginella* require the presence of some soil. The lichen stages may last for many years, but eventually enough soil may be accumulated so that mosses are able to gain a foothold among the lichens. After becoming established, mosses soon replace the lichens. Both mosses and lichens persist in environments that are extremely dry at least part of each year. Collect some dry mosses and li-

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chens and take them into the laboratory. If they are not almost brittle dry, allow them to dry out further. As soon as they are quite dry, place them in a container and add enough water to cover them partially. Place a cover on the container, put it aside, and observe the next day. How are these plants able to survive periods of extreme drought? (19)

Annual Herbaceous Plant Community. Sooner or later, small seed plants become established in the mats among the mosses. In the fall these may be seen only as persisting dried stems. In the spring the general aspect of the community is quite different. For a few weeks extensive areas of shallow soil overlying rocks may be densely covered with annual plants that grow from seeds, produce flowers and fruits within a few weeks, and then die. Locate some communities of these herbaceous annuals. There are only a few of most of these. What advantage is it to these plants to be able to complete their life cycle in a few weeks? (20) Measure the depth of soil in this community. Compare the depth of soil with that of other communities studied. (21)

Perennial Herbaceous Plant Community. As the soil is built up still further, perennial herbaceous plants invade the annual plant community. This community may be recognized readily by the presence of grasses, sedges, and ferns. In regions of relatively low rainfall this may be the final or climax community. In regions of greater rainfall, shrubs follow the herbaceous perennials. Among the herbaceous perennials are certain plants with succulent, fleshy leaves that are capable of holding comparatively large quantities of water. See if you can locate any of these plants. By this time you may have been able to observe three ways in which plants are able to persist year after year in very dry habitats. Can you enumerate the three ways? (22)

The Shrub Community. Locate and observe a community of shrubs. Measure the depth of soil beneath the shrubs. Do you find any herbaceous annuals or perennials beneath the shrubs which were not found in the communities previously studied? (23) If so, what possible explanation do you have for this difference? (24) Where the rainfall is somewhat limited, the shrub community may be the final or climax stage in plant succession.

The Tree Community. Where the annual rainfall is great enough to support a forest, shrubs build up the soil still further and in other ways establish conditions suitable for the development of the tree stage in succession. Observe a tree community which seems to be growing on shallow soil overlying rocks. How deep is the soil? (25) The first tree species to appear are generally those that require full sunlight in the seedling stage. These are replaced later by species that can germinate and grow in the shade of members of the same species. The final climax tree species are likely to be tolerant of shade. On what basis would you determine whether a particular plant community would properly be considered a climax community? (26) Now examine the shrubs and herbaceous plants growing beneath the trees. How do they differ from those growing in the open? (27) Think of as many ways as you can in which the environment beneath the trees differs from that in the open. Do you observe any animals in the forest not seen elsewhere? (28) If so, can you offer any explanation of their presence here and not elsewhere? (29) You have been able to observe that two highly contrasting environments may eventually undergo changes leading to the same type of climax community. In consideration of these observations, which would you consider more important in determining the climax vegetation of a region, the soil or the climate? (30)

APPENDIX

APPENDIX I
UNITS OF MEASUREMENT

THE METRIC SYSTEM

LINEAR MEASURE

10 millimeters	= 1 centimeter
10 centimeters	= 1 decimeter
10 decimeters	= 1 meter
10 meters	= 1 decameter
10 decameters	= 1 hectometer
10 hectometers	= 1 kilometer

LIQUID MEASURE

10 milliliters	= 1 centiliter
10 centiliters	= 1 deciliter
10 deciliters	= 1 liter
10 liters	= 1 decaliter
10 decaliters	= 1 hectoliter
10 hectoliters	= 1 kiloliter
1000 milliliters	= 1 liter

SQUARE MEASURE

100 square millimeters	= 1 square centimeter
100 square centimeters	= 1 square decimeter
100 square decimeters	= 1 square meter
100 square meters	= 1 square decameter
100 square decameters	= 1 square hectometer
100 square hectometers	= 1 square kilometer

WEIGHTS

10 milligrams	= 1 centigram
10 centigrams	= 1 decigram
10 decigrams	= 1 gram
10 grams	= 1 decagram
10 decagrams	= 1 hectogram
10 hectograms	= 1 kilogram
10 kilograms	= 1 quintal
10 quintals	= 1 ton

COMMON CONVERSION FACTORS

A. ENGLISH TO METRIC UNITS

LINEAR

1 inch	= 25.4001 millimeters
1 inch	= 2.54001 centimeters
1 foot	= 0.304 meter
1 yard	= 0.914 meter
1 mile	= 1.609 kilometer

SQUARE

1 square inch	= 645.16 square millimeters
1 square inch	= 6.4516 square centimeters
1 square foot	= 0.092 square meter
1 square yard	= 0.836 square meter
1 square mile	= 2.59 square kilometers
1 acre	= 0.404 hectare

CUBE

1 cubic inch	= 16,387.2 cubic millimeters
1 cubic foot	= 0.0283 cubic meter
1 cubic yard	= 0.764 cubic meter

CAPACITY

1 U.S. liquid ounce	= 29.573 milliliters
1 U.S. liquid quart	= 0.946 liter
1 U.S. liquid gallon	= 3.785 liters
1 U.S. dry quart	= 1.1012 liters
1 U.S. peck	= 8.809 liters
1 U.S. bushel	= 0.352 hectoliter

WEIGHT

(1 troy pound = 0.822 avoirdupois pound)

1 grain	= 0.064
1 avoirdupois ounce	= 28.349 grams
1 troy ounce	= 31.103 grams
1 avoirdupois pound	= 0.453 kilogram
1 troy pound	= 0.373 kilogram
1 long ton	= 1.01 metric tons = 1016.05 kilograms
	(2240 avoirdupois pounds or 2722 troy pounds)
1 short ton	= 0.907 metric ton = 907.18 kilograms
	(2000 avoirdupois pounds or 2430 troy pounds)

KITCHEN MEASURE

1 common tumbler	= ½ pint
60 drops	= 1 teaspoon = 4.9 milliliters
3 teaspoons	= 1 tablespoon = 14.7 milliliters
16 tablespoons	= 1 cup
2 cups	= 1 pint = 0.473 liter

B. METRIC TO ENGLISH UNITS

LINEAR

1 millimeter	= 0.0393 inch
1 centimeter	= 0.3937 inch
1 meter	= 3.280 feet
1 kilometer	= 0.621 mile

CAPACITY

4.9 milliliters	= 1 teaspoon
14.7 milliliters	= 1 tablespoon
30 milliliters	= 1 fluid ounce
1 liter	= 1.056 U.S. liquid quarts

WEIGHT

|	
1 gram	= 0.035 avoirdupois ounce
1 kilogram	= 2.204 avoirdupois pounds

APPENDIX II

TEMPERATURE CONVERSION

It is not difficult to convert temperatures from one scale to the other if you remember the following. A Fahrenheit degree is only ⅝ of a Celsius degree, and 32° F = 0° C (the freezing point of water). If you know the temperature in Fahrenheit degrees, and wish to change to Celsius, you first subtract 32 from the Fahrenheit temperature and take ⅝ of the remainder. To convert from Celsius degrees to Fahrenheit, you reverse the process: Multiply the Celsius temperature by ⅘ and add 32.

The chart on this page can be used to convert temperature quickly by reading across from one scale to the other.

FAHRENHEIT   CELSIUS

boiling point of water

normal body temperature

average room temperature

freezing point of water
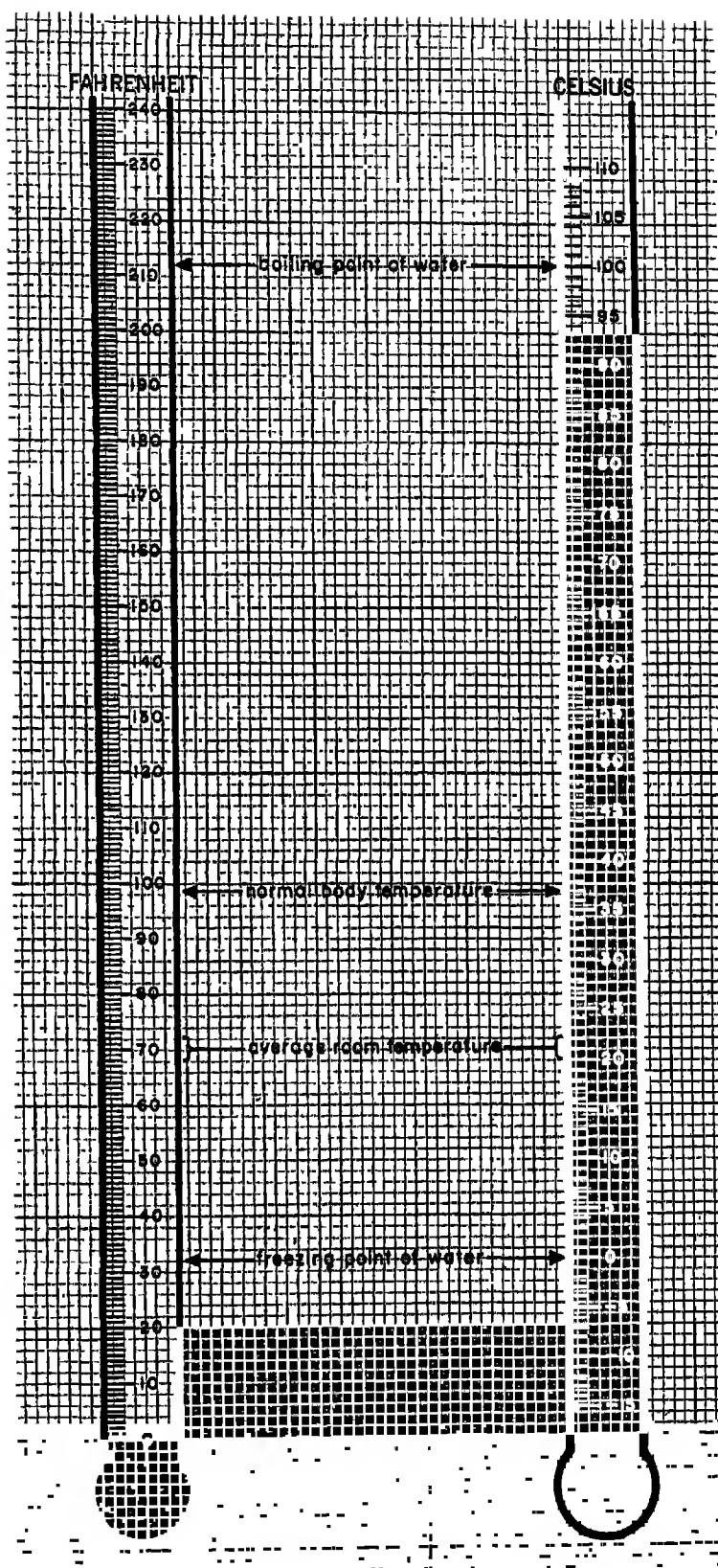
APPENDIX III
USE OF THE CHI-SQUARE METHOD

When the experimental results of scientists differ from the results they expect, an important question arises: How much is the observed difference a matter of accident? For example, suppose that in studying the cross between two kinds of tomato plants it was expected that half the offspring would have green leaves and half would have yellow. (This expectation is based on Mendel's laws and the supposition that a single pair of genes is responsible for the difference between green leaves and yellow leaves.) In one actual experiment it turned out that, of 1240 seedlings, there were 671 with green leaves and 569 with yellow leaves. This is clearly different from the 620 of each kind to be anticipated in a total of 1240. Is it a relatively important difference? Or is it a minor difference that is more a matter of chance than anything else? In this case 620 green-leaved plants were expected and 671 were counted, a difference of 51. Similarly the number of plants with yellow leaves, 569, differs by 51 from the expected 620.

The amount of difference can be expressed in several ways. One way is by expressing it as a percentage of the total: $^{51}/_{1240} = 4.1\%$. A better way of working out a measurement of the amount of difference was invented by Karl Pearson in England in 1900. He called this measure of variation "chi-square." (It is indicated by the Greek letter χ with the square sign, χ^2.) Chi-square is found by squaring each difference between the number expected and the number observed and then dividing by the expected number in each class; and finally adding the quotients together. The difference for the green-leaved plants, 51, is squared, making 2601, and then divided by the expected number of 620. The quotient is 4.2. Again, the difference for the yellow-leaved plants, 51, is squared, to give 2601, and divided by 620, to give the quotient, 4.2. Added together, the two quotients come to 8.4, which is the value of chi-square.

$$671 - 620 = 51 \quad 51^2 = 2601 \quad \left.\frac{2601}{620} = 4.2\right\}$$
$$\left.620 - 569 = 51 \quad 51^2 = 2601 \quad \frac{2601}{620} = 4.2\right\} = 8.4 \text{ chi-square}$$

This does not tell us very much by itself. Fortunately, mathematicians have solved an elaborate distribution equation which gives us the information to judge whether any particular chi-square value represents the sort of differences which occur by chance alone very commonly, or by chance alone most uncommonly, or by chance alone at probabilities somewhere between. The following table which shows these relationships allows us to see about how often a given value of chi-square could have been produced *just* by chance.

	Chi-square Value (χ^2)							
	0.0002	0.004	0.016	0.455	1.074	2.706	3.841	6.635
The number of times in 100 that chance alone could have been responsible for the variation	99	95	90	50	30	10	5	1

$$\chi^2 = \text{sum of all } \frac{(\text{expected} - \text{observed})^2}{\text{expected}}$$

From the table we see that a chi-square figure of 8.4 goes beyond the table. This means that there is less than one chance in 100 by chance alone of getting as big a difference as this experiment actually showed. Experience has shown, and it is generally agreed, that when the probability of an event occurring by chance alone is as little as one or even five chances in 100, then that dif-

ference is said to t.   ... are not occurring by
chance alone, but are a ...

How consistently wi... ...ce differences from each
other? If repeated crosses produce abou... ...es from what was expected, it is time
to make a search for a suitable cause for the variation. (In the case of the tomato plants, furth. r
crosses did show about the same results. The difference was caused by a loss of yellow-leaved
plants, constitutionally less sturdy than the others. Fewer of them germinated and lived.)

The same investigator predicted that the F_2 generation of red and yellow tomatoes should
have a 3 : 1 ratio of red to yellow. (The expectation was based on Mendel's laws and the assump-
tions that one pair of genes determined the difference between red and yellow fruit, and that one
allele is dominant over the other.) The red-fleshed had 3629 fruits, and the yellow-fleshed class
had 1176. From a total of 4805 fruits, we might expect to have 3604 red-fleshed and 1201 yellow-
fleshed by the expected 3 : 1 ratio. Is the difference between observed and expected (25 red and
25 yellow) significant and therefore suggestive of a need to search for a suitable cause? Or is the
difference so small that chance alone could account for it?

Again let us calculate the chi-square value for the differences.

$$3629 - 3604 = 25 \quad 25^2 = 625 \quad \frac{625}{3604} = 0.17 \\ 1201 - 1176 = 25 \quad 25^2 = 625 \quad \frac{625}{1201} = 0.52 \Bigg\} = 0.69 \text{ chi-square}$$

Consulting the table, we find that a variation in results yielding a chi-square equal to 0.69
might occur by chance, and by chance alone, as often as a third of the time or more. (0.69 falls
between 0.455 and 1.074, with respective probabilities that 50 times and 30 times in 100, chance
alone was responsible for the differences.) We conclude that in this experiment the differences
are not significant and that the results do not demand some other theory for explanation. The
difference was considered to be small enough so that chance alone could account for it. To be
sure, *the fact that the results agreed with the theory does not prove that the theory is right.* Some
other theory might lead to expectations that would agree with the results just as well, or even
better. *All we have shown is that our observations are consistent with the theory. It fits the facts.*

When more than two classes occur in the results, it is necessary to use an expanded table to
find the probability that chance alone could have been responsible a certain number of times in
100. Such a table will be needed when we want to consider the significance of results where three
or four classes, all but one independent, are in some kind of ratio, such as the 9 : 3 : 3 : 1 ratio for
the F_2 generation of a dihybrid cross.

When pink-flowered four-o'clocks are crossed, it is expected that the offspring will turn out to
be red- , pink- , and white-flowered in a 1 : 2 : 1 ratio. An experimenter made the cross and found
that he had 66 red-flowered plants, 115 pink-flowered plants and 55 white-flowered plants.

A calculation for chi-square gave a value of 1.18. Consulting the following table, it can be
seen that over half the time (between 50 and 95 times in 100) chance alone could account for the
difference between the observed and expected numbers in the three classes. This is not a signifi-
cant difference, and the experimenter concluded that his results agreed with the 1 : 2 : 1 ratio.
This ratio is found when a single pair of genes determines the trait but neither allele is dominant
over the other. He therefore concluded that, in the absence of a better theory, there was a genetic
basis for these differences in flower color in four-o'clocks.

Chi-square for four classes	0.115	0.352	2.366	3.665	4.642	6.251	7.815	11.341
Chi-square for three classes	0.020	0.103	1.386	2.408	3.219	4.605	5.991	9.210
Chi-square for two classes	0.0002	0.004	0.455	1.074	1.642	2.706	3.841	6.635
The number of times in 100 that chance alone could have been responsible for the variation	99	95	50	30	20	10	5	1